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N-BUTYLIDENE CHLORIDE, A NEW DRUG FOR THE TREATMENT OF EQUINE STRONGYLIDOSIS[1]

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PREVIOUS INVESTIGATIONS

In critical tests made with a large number of chlorinated alkyl hydrocarbons for the purpose of studying the relationship between their anthelmintic efficacy and their chemical structure, Wright and Schaffer (*14*)[2] found that n-butylidene chloride was 100 per cent effective for the removal of ascarids and 95 per cent effective for the removal of hookworms from dogs.

Normal butylidene chloride, $CH_3.CH_2.CH_2.CHCl_2$, is obtained by the action of phosphorus pentachloride on n-butyric aldehyde. For the present experiments a high-grade, commercially prepared product was washed first with a 2 per cent sodium bicarbonate solution and then with water, after which it was dried and distilled. Thus purified, the n-butylidene chloride was a clear, colorless, sweet-tasting liquid with a pleasant odor and possessed the following constants: Boiling point, 115° to 116.5° C. (760 mm.); specific gravity, 1.084 (25°/25°); solubility, 1 gm. in 2,000 gm. of water.

Normal butylidene chloride was found by Wright and Schaffer (*14*) to be well tolerated by dogs in doses up to 10 c. c. per kilogram of body weight. It produced only salivation and a slight muscular trembling, the animals returning to normal within a few hours. In dogs, n-butylidene chloride does not produce the central necrosis of the liver which commonly follows the administration of chlorinated hydrocarbons such as carbon tetrachloride and some of those investigated by Wright and Schaffer.

From the results obtained with n-butylidene chloride on dogs, there was reason to believe that the use of this compound might prove to be an effective treatment for strongylidosis of horses, perhaps better than chenopodium, the drug most commonly used but which has been objected to because of its toxicity. Because of the fact that n-butylidene chloride is less soluble than carbon tetrachloride (solubility 1: 1,250), it was believed that a greater proportion of the n-butylidene chloride might possibly be carried into the caecum and colon, the site of strongyle infestation in horses. The fact that n-butylidene chloride in large doses is well tolerated by dogs and is

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[2] Reference is made by number (italic) to Literature Cited, p. 302.

not followed by severe liver lesions in such animals indicated the possibility that it might safely be administered to horses, in relatively large doses. Accordingly, in the tests reported in this paper doses of 0.207 to 0.333 c. c. per kilogram of body weight were given. These were followed by no deleterious effects.

Of the chlorinated alkyl hydrocarbons, only two, carbon tetrachloride and tetrachlorethylene, have been tested on horses. Hall (*4*) tested carbon tetrachloride alone and also a mixture of carbon tetrachloride and carbon disulphide. He found that carbon tetrachloride alone in doses of 25 to 50 c. c. was partially effective in removing bots, entirely effective in removing the ascarids present in the two test animals that had these parasites, entirely effective in removing strongyles, and partially effective against cylicostomes.

De Blieck and Baudet (*1*), using somewhat larger doses than those employed by Hall, confirmed Hall's results with carbon tetrachloride. Demnitz (*2*), using a dose of 100 gm., carried out tests with carbon tetrachloride on six horses. One of the animals died after treatment and the others were severely intoxicated. Demnitz concluded that carbon tetrachloride was effective but unsafe for horses. However, he administered the drug by passing the stomach tube only one-third the length of the esophagus; the fatality and the intoxications may possibly have resulted from aspiration of the carbon tetrachloride into the trachea and lungs.

Shul'ts and Raevskaia (*12*) reported a series of experiments with carbon tetrachloride on horses. They recommended a routine dose of 150 to 200 c. c. for strong mature animals and stated that the smaller doses recommended by American investigators are not sufficiently effective. They concluded that at best the treatment with carbon tetrachloride can be expected only to lower the degree of infestation and that complete elimination of the strongyles can not be accomplished with this drug.

After treating a considerable number of animals in the clinic of the Berlin Veterinary College, Neumann-Kleinpaul and Pelckmann (*8*) gave, as symptoms produced by the administration of carbon tetrachloride, the following: Apathy, anorexia, increased thirst, colicky symptoms, tremor, bloating, evidences of pain, fluctuating temperature, variation in the pulse and respiration rates, cough, diarrhea, icterus, and the appearance of albumin in the urine. These investigators found that the degree of toxicity is not always in proportion to the size of the dose of carbon tetrachloride. Furthermore, they demonstrated that the blood-calcium level, computed from the daily average, sank from 4 to 14.56 per cent below its original level after treatment with carbon tetrachloride in doses of 0.15 to 0.33 gm. per kilogram of body weight, indicating that the relation of calcium to the toxicity of carbon tetrachloride in horses is substantially the same as in dogs, as demonstrated by Minot (*7*). Neumann-Kleinpaul and Pelckmann found also that the toxic symptoms produced by carbon tetrachloride in horses, especially variations in the temperature, pulse, and respiration, occur in the main at the time of the declining blood-calcium level, although the duration and intensity of the toxic symptoms correspond in no evident way with the degree of decline in the blood-calcium level. By feeding a sufficient quantity of calcium chloride starch (a proprietary preparation) before the administration

of carbon tetrachloride, these authors were able to prevent entirely the reduction of the blood-calcium level and to diminish the toxic symptoms commonly observed by them after the use of the drug. The feeding of ultra-violet-treated vitamin yeast before the administration of carbon tetrachloride diminished the reduction of the blood-calcium level and lessened considerably the duration and intensity of all toxic symptoms.

Grassnickel (*3*) found that the bilirubin content of the blood of the animals used in the experiments of Neumann-Kleinpaul and Pelckmann was increased in every case after carbon tetrachloride administration and that the increase was 1.2 to 4 times the normal bilirubin content. In 9 of 15 horses the strongest intoxication reactions corresponded with the high point of the bilirubin level, or the high point of the bilirubin level fell on the day of the toxic symptoms. The lowest blood-calcium level coincided with the highest bilirubin level in two of the animals, although the reduction of the blood-calcium level and the rise in the bilirubin content stood in no evident dependent relationship to each other.

In substance, the investigations of the above-mentioned authors indicate that the horse is no less susceptible to carbon tetrachloride intoxication than are other animals and that, as with other animals, before the administration of carbon tetrachloride the diet should be regulated in such a way as to provide a high calcium intake in order to offset lowering of the blood-calcium level and the retention of guanidine.

Schlingman (*10*) tested tetrachlorethylene on three horses and found that the drug, when given in doses of 0.066 to 0.15 c. c. per kilogram of body weight, produced drowsiness and, in one animal, marked incoordination of movement. The anthelmintic was about 50 per cent effective against bots. Schlingman states that none of the horses passed any roundworms and that only one roundworm was found post-mortem. As the term "roundworm" is not specific, it is not clear what species of parasite resisted the action of the drug; probably it was an ascarid. Schlingman's tests, therefore, throw no light on the efficacy of tetrachlorethylene for nematode parasites of the horse. Raffensperger, in a paper as yet unpublished, has reported on critical tests of tetrachlorethylene alone and in mixtures with oil of chenopodium for parasites of the horse.

EXPERIMENTAL PROCEDURE

With the exception of the first experiment reported in this paper, where visual examination of the feces was made after treatment, the method employed was that developed by Hall and commonly used in all anthelmintic work carried out in the Zoological Division. This method consists of screening all feces passed after the administration of the anthelmintic in order to recover all parasites removed by the treatment, the slaughter of the animal after a suitable number of days, and the screening of the contents of the digestive tract to recover worms not removed by the treatment.

RESULTS OF EXPERIMENTS

EXPERIMENT 1

Horse No. 1, an 8-months-old colt weighing 282 kgm. (620 pounds), was fasted 24 hours and dosed by stomach tube with 0.21 c. c. of n-butylidene chloride per kilogram of weight, a total of 60 c. c., in 480 c. c. of raw linseed oil. There was no reaction to the treatment. The animal passed the following parasites:

	Number and kind of worms passed
First day	2 immature ascarids, 19 cylicostomes, 1 oxyurid.
Second day	2 cylicostomes, 1 bot.
Third day	7 immature ascarids, 1,566 cylicostomes, 1 oxyurid.
Fourth day	2 immature ascarids, 208 cylicostomes, 4 *Poteriostomum ratzii*.
Fifth day	1 immature ascarid, 24 cylicostomes, 2 *P. ratzii*, 1 bot.
Sixth day	None.
Total	12 immature ascarids, 1,819 cylicostomes, 6 *P. ratzii*, 2 oxyurids, 2 bots.

The animal was killed 17 days after the administration of the anthelmintic. No fecal examination for worms was made between the sixth and seventeenth days, but presumably all worms removed by the anthelmintic would have been passed by the sixth day. The following parasites were recovered post-mortem:

Location	Number and kind of worms found post-mortem
Stomach	266 *Gastrophilus intestinalis*, 59 *G. nasalis*.
Small intestine	139 immature ascarids.
Caecum and large colon.	13 *Strongylus vulgaris*, 1 immature ascarid, 1 *Triodontophorus intermedius*, 13 *T. tenuicollis*, 449 cylicostomes, hundreds[3] of oxyurid larvae, and large numbers[3] of *Probstmayria vivipara*.
Small colon	3 immature ascarids.

A large ulcer in the wall of the large colon contained 7 *Triodontophorus tenuicollis*. There were 30 larvae of *Strongylus vulgaris* in different stages of development in a large aneurism of the anterior mesenteric artery.

If the 1 immature ascarid in the caecum and the 3 in the small colon are considered as having been removed by the anthelmintic, the treatment was 11 per cent effective against ascarids and 0 per cent effective against strongyles, *Triodontophorus* spp., and *Probstmayria vivipara*. The anthelmintic removed 80.2 per cent of the cylicostomes, less than 1 per cent of the oxyurids, and 1 per cent of the bots. However, as all the ascarids found in the animal were immature and the colt was not killed until 17 days after the administration of the anthelmintic, it can not be safely assumed that the drug removed only 11 per cent of the ascarids, since some or many of those remaining may have reached the intestinal tract after the treatment was given. The same may be said for *Strongylus vulgaris*. Therefore, this particular test failed to give accurate information as to the value of n-butylidene chloride against ascarids and strongyles.

Schlingman (*11*) has shown that the administration of fats and oils reduces the efficacy of tetrachlorethylene for dogs, and Hall (*5*)

[3] Owing to the very small size of these worms and the large quantity of fecal material, detailed counting would have involved an unwarranted expenditure of time, especially in view of the apparent low efficiency of the drug against these species.

found that oil reduced the efficacy of carbon tetrachloride, a fact in keeping with the findings of Lamson, Robbins, and Ward (*6*) and Robbins (*9*) that the rate of absorption of carbon tetrachloride and tetrachlorethylene is greatly increased in the presence of fats and oils. It was thought that these results might apply to other chlorinated hydrocarbons and that therefore the simultaneous administration of n-butylidene chloride with raw linseed oil increased the absorption and reduced the anthelmintic efficacy of the drug. For this reason, in the remaining experiments in which oils were used, they were given five hours after the administration of the n-butylidene chloride.

EXPERIMENT 2

Horse No. 2, a 22-year-old gelding weighing 482 kgm. (1,060 pounds), was fasted 24 hours and dosed by stomach tube with 0.207 c. c. of n-butylidene chloride per kilogram of weight, a total of 100 c. c., followed in five hours by 960 c. c. of raw linseed oil. There was no reaction to the treatment; the animal refused feed the day after treatment but the second day consumed the usual quantity. The following parasites were passed:

	Number and kind of worms passed
First day	18 cylicostomes.
Second day	175 cylicostomes.
Third and fourth days	374 cylicostomes, 10 *Strongylus edentatus*, 5 *Triodontophorus tenuicollis*. 2 *Poteriostomum ratzii*, 12 *Craterostomum* spp.
Fifth day	5 *S. edentatus*, 5 cylicostomes.
Sixth day	None.
Total	572 cylicostomes, 15 *S. edentatus*, 5 *T. tenuicollis*, 2 *P. ratzii*, 12 *Craterostomum* spp.

The animal was killed 15 days after the administration of the treatment, and the following parasites were recovered post-mortem:

Location	Number and kind of worms found post-mortem
Stomach	25 *Gastrophilus intestinalis*, 81 *G. nasalis*.
Rectum	1 *G. hemorrhoidalis*.
Caecum	1 *Strongylus vulgaris*.

Each of two abscesses in the wall of the double colon contained one *Strongylus vulgaris*. An aneurism was found in the anterior mesenteric artery, but it contained no strongyle larvae. The treatment was 100 per cent effective against cylicostomes, *S. edentatus*, *Poteriostomum ratzii*, *Triodontophorus tenuicollis*, and *Craterostomum* spp. It failed to remove the one specimen of *S. vulgaris*, which, however, might have entered the intestinal tract after the administration of the treatment, as the horse was not killed until 15 days after treatment. The treatment was entirely ineffective for the removal of bots.

EXPERIMENT 3

Horse No. 3, a 2-year-old colt weighing 324 kgm. (712 pounds), was fasted 24 hours and dosed by stomach tube with 0.309 c. c. of n-butylidene chloride per kilogram of weight, a total of 100 c. c., followed in five hours by 480 c. c. of raw linseed oil, also given by

stomach tube. There was no reaction to the treatment. The following parasites were passed:

	Number and kind of worms passed
First day	294 cylicostomes, 1 oxyurid, 1 bot.
Second day	106 cylicostomes, 1 *Strongylus* sp.,[4] 2 oxyurids, 2 bots.
Third day	78 cylicostomes, 3 *Poteriostomum imparidentatum*, 1 *Craterostomum* sp., 1 *Oesophagodontus robustus*.
Fourth day	19 cylicostomes.
Fifth day	None.
Total	497 cylicostomes, 1 *Strongylus* sp., 3 oxyurids, 3 bots, 3 *P. imparidentatum*, 1 *Craterostomum* sp., and 1 *O. robustus*.

The animal was killed on the seventh day after treatment and the following parasites were collected post-mortem:

Location	Number and kind of worms found post-mortem
Stomach	Large numbers of *Gastrophilus intestinalis* and *G. nasalis* present but not counted.
Small intestine	None.
Caecum	2 cylicostomes, 12 *Strongylus vulgaris*, 1 *S. edentatus*.
Double colon	10 cylicostomes, 1 *Poteriostomum imparidentatum*, 4 *oxyurids*.

The efficacy of the treatment was as follows: 97 per cent for cylicostomes, 7 per cent for *Strongylus* spp., 75 per cent for *Poteriostomum imparidentatum*, 100 per cent for *Oesophagodontus robustus*, 43 per cent for oxyurids, and probably less than 1 per cent for bots.

In an aneurism in the anterior mesenteric artery there were several agamic specimens of *Strongylus vulgaris*. Here, again, it was not possible to determine whether specimens of *S. vulgaris* unaffected by the anthelmintic entered the caecum after the administration of the drug and were found there post-mortem, although in view of the number found it would be reasonable to assume that at least some of these worms were present as adult forms in the large intestine at the time of treatment.

EXPERIMENT 4

Mule No. 430, a female about 18 years of age, weighing 295 kgm. (649 pounds), was fasted 36 hours and dosed by stomach tube with 0.3 c. c. of n-butylidene chloride per kilogram of weight, a total of 88.5 c. c., followed in five hours by 180 c. c. of castor oil in 540 c. c. of liquid petrolatum, also given by stomach tube. The animal did not consume all the feed the next day, but on the second day after treatment it ate normally and continued to do so thereafter. The following parasites were passed:

	Number and kind of worms passed
First day	None.
Second day	1 *Poteriostomum ratzii*, 636 cylicostomes, 1 *Strongylus equinus*.
Third day	1 *P. ratzii*, 966 cylicostomes, 4 *S. vulgaris*, 2 *S. equinus*, 2 *S. edentatus*.
Fourth day	1 *P. ratzii*, 220 cyclicostomes, 1 *S. vulgaris*, 1 *S. edentatus*.
Fifth day	3 *P. ratzii*, 80 cylicostomes.

The animal was killed on the fifth day after treatment. The following parasites were collected from the small colon and must be

[4] Specific identification was not possible because of maceration.

regarded as having been passed out and therefore credited as removed by the anthelmintic: 12 *Poteriostomum ratzii*, 72 cylicostomes, 1 *Strongylus vulgaris*, and 1 *S. edentatus*.

Total worms passed: 18 *Poteriostomum ratzii*, 1,974 cylicostomes, 6 *Strongylus vulgaris*, 3 *S. equinus*, and 4 *S. edentatus*. The following parasites found post-mortem were not affected by the anthelmintic:

Location	Number and kind of worms found post-mortem
Stomach	22 *Gastrophilus intestinalis*, 7 *G. nasalis*.
Small intestine	7 *G. nasalis*.
Caecum	1 *Strongylus vulgaris*.
Large colon	29 *Poteriostomum* spp., 7,086 cylicostomes, 3 *S. vulgaris*, 3 *S. equinus*, 5 *S. edentatus*, 1 oxyurid larva.

The efficacy of the treatment was as follows: 37 per cent for *Poteriostomum* spp., 21.8 per cent for cylicostomes, 60 per cent for *Strongylus vulgaris*, 50 per cent for *S. equinus*, 44.4 per cent for *S. edentatus* (or 52 per cent for all *Strongylus* spp.), 0 per cent for oxyurids, and 0 per cent for bots. One *S. vulgaris* found in the large colon was embedded in the wall of that organ, the head end projecting for a distance of 6 mm. above the mucosa. Obviously no anthelmintic could have removed this specimen. An aneurism in the anterior branch of the anterior mesenteric artery contained 3 larvae of *S. vulgaris*, and a second aneurism in the right branch of the anterior mesenteric artery contained 15 larvae of *S. vulgaris*.

In the animals used in the first three experiments, n-butylidene chloride failed completely to remove any specimens of *Strongylus vulgaris*, but in the present experiment the drug removed a larger percentage of this species than of any other. This fact indicates that *S. vulgaris* is not necessarily resistant to the action of n-butylidene chloride, although it might be inferred from the three previous experiments that it is. Apparently the drug will remove a majority of these forms when they exist as adults in the lumen of the intestinal tract at the time the anthelmintic is administered.

The results against cylicostomes in the present experiment are at variance with those obtained in the three previous tests. The purgative in this test was different from that used in the three previous ones, a fact which may have influenced the efficacy of the n-butylidene chloride against cylicostomes. This point is more fully discussed later. It will be noted that the drug removed all these parasites from the caecum but left a large number in the double colon. This circumstance suggests that by the time it reached the double colon the drug was so diluted that it was not present in sufficient concentration to act on the worms.

EXPERIMENT 5

Horse No. 466, a 10-year-old mare weighing 459 kgm. (1,010 pounds), was fasted 36 hours and dosed with 0.3 c. c. n-butylidene chloride per kilogram of weight, a total of 137.7 c. c., administered in capsules, followed immediately by 1 ounce of aloes in capsule. Several capsules containing n-butylidene chloride were broken in the mouth, but the animal suffered no reaction from these accidents.

There was no reaction to the treatment. The following parasites were passed:

	Number and kind of worms passed
First day	8 cylicostomes.
Second day	332 cylicostomes.
Third day	1,496 cylicostomes, 1 oxyurid, 5 *Strongylus edentatus*.
Fourth day	800 cylicostomes, 1 *S. vulgaris*, 6 *S. edentatus*.
Fifth day	644 cylicostomes.

The animal was killed on the fifth day after treatment; 640 cylicostomes were recovered from the small colon and must be considered as having been removed by the anthelmintic.

Total worms passed: 3,920 cylicostomes, 11 *Strongylus edentatus*, 1 *S. vulgaris*, and 1 oxyurid. Parasites not affected by the anthelmintic were recovered post-mortem, as follows:

Location	Number and kind of worms found post-mortem
Stomach	2 *Gastrophilus nasalis*, 2 *G. intestinalis*.
Small intestine	1 *G. nasalis*.
Caecum	256 cylicostomes.
Large colon	14,360 cylicostomes, 8 *Strongylus edentatus*, 12 *Tricdontophorus serratus*, 2 *Poteriostomum ratzii*, 1 oxyurid.

The efficacy of the treatment was as follows: 100 per cent for *Strongylus vulgaris*, 57.9 per cent for *S. edentatus* (or 60 per cent for all *Strongylus* spp.), 21.2 per cent for cylicostomes, 0 per cent for *Poteriostomun ratzii*, 50 per cent for oxyurids, 0 per cent for *Triodontophorus serratus*, and 0 per cent for bots.

Aloes did not prove to be an advantageous purgative, as it gave no better supplementary action to the anthelmintic than did the castor-oil liquid-petrolatum mixture used in the previous experiment. As in the previous experiment, the anthelmintic removed most of the cylicostomes from the caecum but apparently failed to reach those in the colon. The large number of cylicostomes recovered from this animal is remarkable.

EXPERIMENT 6

Horse No. 4, an aged mare weighing 432 kgm. (950 pounds), was fasted 30 hours and dosed by stomach tube with 0.317 c. c. of n-butylidene chloride per kilogram of weight, a total of 137 c. c., followed in five hours by 960 c. c. of raw linseed oil. There was no immediate reaction to the treatment, but one hour later the mare was sweating profusely and persisted in lying down. The pulse was 62 and the respiration 40 per minute. Two hours after the treatment the mare had returned to normal, the pulse being 40 and the respiration 10. The following parasites were passed:

	Number and kind of worms passed
First day	356 cylicostomes, 1 bot.
Second day	280 cylicostomes.
Third day	235 cylicostomes, 1 *Strongylus edentatus*, 1 *Poteriostomum ratzii*, 3 bots.
Fourth day	3 cylicostomes, 2 bots.
Fifth and sixth days.	None.
Seventh day	1 bot.
Total	874 cylicostomes, 1 *S. edentatus*, 1 *P. ratzii*, 7 bots.

The mare was killed 11 days after treatment, and the following parasites were recovered post-mortem:

Location	Number and kind of worms found post-mortem
Stomach	8 *Gastrophilus intestinalis.*
Caecum	1 *Strongylus vulgaris.*
Colon	None.

The efficacy of the treatment was as follows: 100 per cent for cylicostomes, 100 per cent for *Strongylus edentatus*, 0 per cent for *S. vulgaris* (or 50 per cent for all *Strongylus* spp.), 100 per cent for *Poteriostomum ratzii*, and 46.7 per cent for bots.

The fact that the animal was not killed until 11 days after treatment suggests the possibility that the one *Strongylus vulgaris* found post-mortem entered the lumen of the gut subsequent to the administration of the anthelmintic and that the drug should not be charged with the failure to remove this parasite.

EXPERIMENT 7

Horse No. 5, an aged gelding weighing 450 kgm. (990 pounds), was not fasted before dosing and was given 0.333 c. c. of n-butylidene chloride per kilogram of weight, a total of 150 c. c. This was followed in five hours by 960 c. c. of raw linseed oil. There was no reaction to the treatment. The following parasites were passed:

	Number and kind of worms passed
First day	874 cylicostomes, 2 *Strongylus* spp.[5]
Second day	71 cylicostomes, 4 *S. edentatus.*
Third day	4 cylicostomes, 2 *S. edentatus.*
Fourth day	None.
Total	949 cylicostomes, 8 strongyles.

The animal was killed nine days after treatment and the following worms recovered post-mortem:

Location	Number and kind of worms found post-mortem
Stomach	13 *Gastrophilus intestinalis.*
Caecum	None.
Double colon	1 *Triodontophorus tenuicollis.*
Small colon and rectum	None.

There were 3 larvae of *Strongylus vulgaris* in an aneurism of the anterior mesenteric artery. Efficacy of the treatment: 100 per cent for cylicostomes, 100 per cent for *Strongylus* spp., 0 per cent for *Triodontophorus tenuicollis*, and 0 per cent for bots.

EXPERIMENT 8

Horse No. 452, a 12-year-old gelding weighing 482.7 kgm. (1,062 pounds), was fasted 36 hours and given in capsules 0.3 c. c. of n-butylidene chloride per kilogram of weight, a total of 144.8 c. c., followed in five hours by 960 c. c. of raw linseed oil in a drench. There was no reaction to the treatment. The following parasites were passed:

	Number and kind of worms passed
First day	No feces.
Second day	64 cylicostomes.[6]

[5] Could not be specifically identified for the reason that the anterior part was missing in each instance.
[6] The feces on this day were dry and hard and an additional dose of 960 c. c. of raw linseed oil was administered in the afternoon.

Third day..... 548 cylicostomes, 4 *Poteriostomum ratzii*.
Fourth day..... 32 cylicostomes.
Fifth day..... No feces.
Total..... 644 cylicostomes, 4 *P. ratzii*.

The animal was killed on the fifth day after dosing. The small colon contained 1 *Poteriostomum ratzii*, and the large colon contained 1 *Strongylus equinus* dead and much disintegrated with the head missing, identification being made by the bursa; these parasites must be considered as having been removed by the anthelmintic. No other parasites were found post-mortem. Efficacy of the treatment: 100 per cent for *Strongylus* spp., cylicostomes, and *P. ratzii*.

OTHER TESTS

Dr. Robert Bardwell, Mereworth Stud, Lexington, Ky., offered to conduct tests with n-butylidene chloride, and a quantity of the compound was forwarded to him. The following is his report of one such test:

> We had a farm foal raised with the other foals which showed a few ascaris and strongyle eggs on fecal examination. After starving her about 16 hours, I gave 50 c. c. The feces were examined for 48 hours but no worms found. The foal was very constipated as a result of the drug and was slightly off feed, although nothing serious was noted. Fecal examination one week after treatment was negative for ascarids, positive for strongyles; at two weeks fecal examination was negative for both. The foal was autopsied but I could find no bots or worms of any kind. As a result of this, I feel that this drug may be very useful.

In Dr. Bardwell's test, n-butylidene chloride apparently removed all ascarids from the test animal, whereas in experiment 1, the drug given with raw linseed oil apparently failed to remove many of the ascarids present. It is probable that mixing raw linseed oil with n-butylidene chloride reduces the efficacy of the anthelmintic against ascarids.

TESTS AGAINST HABRONEMA SPP.

Wright, Bozicevich, and Underwood (*13*), in reporting upon the use of carbon disulphide as a treatment for equine habronemiasis, indicate that carbon disulphide is a very effective treatment for the destruction of *Habronema* spp. and that the efficacy of the drug is augmented when administration is preceded by gastric lavage with a 2 per cent solution of sodium bicarbonate. N-butylidene chloride was tested on three animals infested with *Habronema* spp.

A gray gelding, about 15 years old and weighing approximately 1,200 pounds, was dosed at 8.25 a. m. with 60 c. c. of n-butylidene chloride in capsules. The animal appeared uneasy after the treatment and moved about considerably. At 9.15 a. m. the horse was killed. Although the animal was said to have been fasted for 12 hours before treatment, the stomach was found to contain about 3 liters of feed. The organ was immediately examined and the mucosa found to be slightly inflamed. The odor of n-butylidene chloride was pronounced. One *Habronema muscae* was found; this specimen was alive.

A roan mare, about 15 years old and weighing about 800 pounds, was dosed at 8.20 a. m. with 60 c. c. of n-butylidene chloride in capsules. There was no reaction to the treatment. The mare was killed at 9.50 a. m. The mucosa of the stomach was slightly inflamed. Nine *Habronema muscae* were found in the organ, 4 being alive and 5 dead. The percentage of efficiency was therefore 55.5.

A black gelding, about 15 years old, and weighing about 1,100 pounds, was dosed with 60 c. c. of n-butylidene chloride in capsules at 8.50 a. m. There was no reaction to the treatment. The animal was killed at 11.10 a. m. Although the animal was said to have been fasted for 12 hours before treatment, the stomach was engorged with food. One hundred and nine *Habronema microstoma* were recovered, of which 3 were alive and 106 dead. In this test, n-butylidene chloride was 97.2 per cent effective for the destruction of *H. microstoma*.

In the last test the interval of time between dosing and slaughter of the animal was greater than in the two other tests, and it is possible that the additional time gave the anthelmintic greater opportunity to act on the parasites present in the stomach. It would appear from these tests that n-butylidene chloride is partially effective against *Habronema* spp.; no doubt the efficacy of the drug would be materially increased if it were preceded by gastric lavage with an alkaline solution as has been shown to be the case with carbon disulphide. It has been impossible to determine this point because of the unavailability of suitable test animals.

DISCUSSION OF RESULTS

In these tests, n-butylidene chloride administered in doses of 0.207 to 0.333 c. c. per kilogram of body weight, followed in five hours by 960 c. c. (1 quart) of raw linseed oil for a 1,000-pound animal (experiments 2, 6, 7, and 8), gave good results against *Strongylus* spp., cylicostomes, and *Poteriostomum* spp. In experiment 2, the drug removed 93.8 per cent of all *Strongylus* spp., all of the cylicostomes, and all *Poteriostomum* spp. In experiment 6, 50 per cent of the *Strongylus* spp., all of the cylicostomes, and all *Poteriostomum* spp. were removed. In experiment 7, all *Strongylus* spp. and cylicostomes were removed. In experiment 8, all *Strongylus* spp., cylicostomes, and *P. ratzii* were removed.

The administration of n-butylidene chloride in raw linseed oil in experiment 1 resulted in reduced efficacy for the anthelmintic, as none of the *Strongylus* spp. present were removed and only 80.2 per cent of the cylicostomes. In experiment 3, in which the drug was followed in five hours by 480 c. c. of raw linseed oil for a 712-pound animal it is apparent that the dose of linseed oil was insufficient to insure adequate purgation and possibly the reduced efficacy in this test is correlated with the use of an inadequate amount of purgative.

A castor-oil mineral-oil mixture, as used in experiment 4, also failed to provide adequate purgation, and apparently this mixture is not advantageous for use as a purgative in connection with n-butylidene chloride. The results against cylicostomes were disappointing in this experiment and also in experiment 5, in which aloes was used as a purgative, although respective efficacies of 52 per cent and 60 per cent were obtained for *Strongylus* spp. N-butylidene chloride in these two experiments removed nearly all worms from the caecum but failed to have an adequate effect on those in the double colon, a fact which suggests that the purgative used was not of a nature suitable to carry the anthelmintic into the double colon in sufficient quantity or concentration to act effectively on the worms located there. It may be

assumed from a theoretical standpoint that most of the n-butylidene chloride was absorbed before it reached this portion of the gut.

It will be noted that extremely variable results were obtained with n-butylidene chloride against *Strongylus* spp., the percentage of efficacy ranging from 0 to 100. As these parasites wander as larvae into the tissues, develop to fully grown agamic forms at points outside the lumen of the large intestine, and ultimately return to the lumen and promptly assume the appearance and character of mature worms, it can not be said with certainty that strongyles found post-mortem 5 to 10 days after treatment were present in the gut at the time of treatment. No doubt this return of strongyles to the lumen of the gut is a more or less continuous process, especially in the case of *S. vulgaris*, the agamic forms of which are found in aneurisms usually present in the anterior mesenteric artery or its branches. As such aneurisms were present in nearly all the test animals used in these experiments, it is possible, and even probable, that the ineffectiveness of n-butylidene chloride against *S. vulgaris* in some of the tests was in part due to the fact that adult forms found in the large intestine at necropsy had reached that location after the administration of the anthelmintic.

Of one thing we can at least be sure, that n-butylidene chloride is as effective as indicated in these tests; probably it is much more effective against *Strongylus* spp. than some of the tests would indicate. In this connection Hall (*4, p. 521*) reports:

> * * * We are, therefore, safe in concluding that the drugs which remove all or almost all of these worms are effective, that those which remove few or none and leave many behind are ineffective, and that those removing any given fraction based on the number collected from the manure and the number found post-mortem are substantially as effective as the indicated fraction stated in percentage figures, with the qualifying statement that they may be somewhat more effective than the findings indicate.

ASCARIDS

The colt used in experiment 1 was the only test animal infested with ascarids. Only 11 per cent of these worms were removed. However, as all ascarids found post-mortem were immature and the animal was not killed until 17 days after the administration of the anthelmintic, it is possible that many of these worms reached the small intestine after the treatment was given. In this test, n-butylidene chloride was given in raw linseed oil, a method which probably greatly reduced its efficacy. Evidently this test does not afford a basis for drawing final conclusions, as in Dr. Bardwell's test an efficacy of 100 per cent was apparently obtained against ascarids.

BOTS

The results against bots were very unsatisfactory. In experiment 1, only 1 per cent of the bots were removed and in experiment 6, 46.7 per cent. In five other tests, n-butylidene chloride failed to remove any of the bots present. It is apparent that the drug possesses little efficacy against these parasites.

HABRONEMA SPP.

N-butylidene chloride failed to kill the one specimen of *H. muscae* present in one animal but showed an efficacy of 55.5 per cent against this species in another animal. In a third test, the drug proved to be 97.2 per cent effective for the destruction of *H. microstoma*. In

this test, a greater period of time elapsed between treatment and killing of the animal, a circumstance which no doubt permitted the drug more time to act on the parasites. Probably the use of a preliminary gastric lavage would increase the efficacy of n-butylidene chloride for *Habronema* spp.

STRONGYLUS SPP.

In experiments 1, 2, 3, and 6, n-butylidene chloride failed to remove any specimens of *S. vulgaris;* the drug removed 60 per cent of this species in experiment 4 and 100 per cent in experiment 5. The efficacy of n-butylidene chloride against *S. edentatus* varied from 44.4 per cent in experiment 4 to 100 per cent in experiments 2, 6, and 7. In experiment 4, the drug removed 50 per cent of *S. equinus* and in experiment 8, 100 per cent. The efficacy against all species of Strongylus varied from 0 to 100 per cent, the most consistent being in those cases where n-butylidene chloride was followed in five hours by a dose of raw linseed oil adequate for the individual animal.

CYLICOSTOMES

In experiments 2, 6, 7, and 8, where the anthelmintic was followed in five hours by an adequate dose of raw linseed oil, 100 per cent effectiveness was obtained against cylicostomes. In experiment 3, where the dose of linseed oil was not sufficient, the treatment removed 97 per cent of these forms. The administration of n-butylidene chloride in raw linseed oil gave only 80.2 per cent efficacy in experiment 1. In experiment 4, where a castor-oil mineral-oil mixture was used, and in experiment 5, where aloes was used, the results against cylicostomes were decidedly inferior. When properly administered, n-butylidene chloride apparently can be depended upon to remove all or nearly all cylicostomes.

TRIODONTOPHORUS SPP.

These parasites were present in only four of the eight test animals. In experiment 2, n-butylidene chloride removed all of the *T. tenuicollis* present. It failed to remove any of this form in experiments 1 and 7 or any of the specimens of *T. serratus* present in horse No. 466 (experiment 5).

POTERIOSTOMUM SPP.

In experiments 1, 2, 6, and 8, n-butylidene chloride removed all *Poteriostomum* spp. present. It was only 75 per cent effective in experiment 3, where an adequate dose of raw linseed oil was not given. With a castor-oil mineral-oil mixture in experiment 4, the drug removed only 37 per cent of these forms, and in experiment 5, where aloes was used as a purgative, the results were negative.

PROBSTMAYRIA VIVIPARA

Probstmayria vivipara was present in only one animal (experiment 1); n-butylidene chloride administered in raw linseed oil failed to remove any of these forms.

CRATEROSTOMUM SPP.

The drug removed all *Craterostomum* spp. present in test animals in experiments 2 and 3.

OESOPHAGODONTUS ROBUSTUS

N-butylidene chloride removed the one specimen of *O. robustus* present in the test animal in experiment 3.

OXYURIDS

In experiment 1, n-butylidene chloride removed 2 oxyurids but left hundreds of larvae, being less than 1 per cent effective. In experiment 3, the drug was 43 per cent effective, and in experiment 5 it was 50 per cent effective. Followed by a castor-oil mineral-oil mixture in experiment 4, n-butylidene chloride failed to remove the one larva present. These results are not conclusive, as this species was not present in any of the animals in which n-butylidene chloride was followed by a dose of raw linseed oil sufficient for adequate purgation, a method of treatment which might have been productive of better results if the efficacy of the drug can be judged by its effects against other parasites when it was administered in this way.

MICROSCOPICAL PATHOLOGY

The microscopical pathology of the liver and kidneys of three of the animals used in these tests was studied. Portions of these organs were obtained immediately after slaughter and fixed in 10 per cent formalin solution. The sections were stained with haematoxylin and eosin. A study of these sections indicated the following:

MULE No. 430 (dosed with 0.3 c. c. of the drug per kilogram of weight).—Liver: Chronic interstitial hepatitis; generalized cloudy swelling of more than moderate intensity; a few large and small fat droplets in the cells of some lobules but distribution not uniform and no marked fatty degeneration. Kidney: Considerable hyperemia; other lesions subacute or chronic, consisting of interstitial nephritis with extensive round-cell infiltration and beginning fibroblastic formation; glomerulonephritis with thickening of the glomerular capsule.

HORSE No. 466 (dosed with 0.3 c. c. per kilogram of weight).—Liver: Slight cellular hepatitis; generalized cloudy swelling of only moderate intensity and less in degree than that exhibited by the previous animal; a few small fat droplets in cells of some lobules, these droplets being situated mainly in the periphery of the lobules; slight eosinophilia. Kidney: Considerable hyperemia; marked chronic interstitial nephritis.

HORSE No. 452 (dosed with 0.3 c. c. per kilogram of weight).—Liver: Cloudy swelling of about the same degree as that exhibited by mule No. 430; cytoplasm of liver cells granular and poorly stained; considerable accumulation of large and small fat droplets not uniformly distributed but more numerous in some lobules than in others; in some lobules fatty inclusions from the center to the periphery of the lobules; slight cellular hepatitis and slight leucocytosis. Kidney: Severe generalized cloudy swelling; cytoplasm of tubular epithelium granular and disintegrated, the lumen of the tubules containing epithelial débris, some of which seemed to be partially degenerated into hyalinelike material; marked hemorrhages into the tubules and glomeruli; slight chronic interstitial nephritis.

From the above study it may be said that n-butylidene chloride, in doses of 0.3 c. c. per kilogram of body weight, produces in the horse a moderately severe cloudy swelling of the liver with a slight deposition of fat, which in no case is well marked or extensive. The acute kidney lesions in the first two animals were confined to a hyperemia, but in horse No. 452 the lesions were more pronounced. The liver of this animal also had a greater accumulation of fat than did the livers of the other two animals. Horse No. 452 was apparently suffering from constipation at the time of treatment; no feces were passed on the day after treatment, and it was necessary to give an additional dose of purgative on the afternoon of that day. It seems

reasonable to assume that the inadequate elimination in this animal resulted in an increased absorption of the anthelmintic from the intestinal tract with the production in the liver and kidneys of lesions more severe than those in the two animals in which proper elimination was obtained. It is probable, therefore, that in those cases where the n-butylidene chloride would be rapidly eliminated from the intestinal tract by the action of the purgative, damage to the liver and kidneys would not be sufficient to militate against the use of this drug as an anthelmintic for the horse. In fact, in this respect n-butylidene chloride seems to possess some advantage over some anthelmintics now in use.

SUMMARY

N-butylidene chloride administered to four animals at a dose rate of 0.207 to 0.333 c. c. per kilogram of body weight and followed in five hours by 960 c. c. of raw linseed oil for a 1,000-pound animal resulted in efficacies of 50 per cent, 94 per cent, 100 per cent, and 100 per cent, respectively, against *Strongylus* spp., and of 100 per cent for cylicostomes in all four animals. The anthelmintic removed all of the *Poteriostomum* spp. present in the three animals that harbored these parasites.

In other tests the efficacy of n-butylidene chloride for the above-mentioned parasites was considerably lowered where the drug was administered in raw linseed oil or where it was followed by aloes or by a castor-oil mineral-oil mixture.

When administered in raw linseed oil, n-butylidene chloride was relatively ineffective for the removal of ascarids, but when given without oil the drug was apparently effective for the removal of this species.

N-butylidene chloride, administered to three animals weighing from 800 to 1,200 pounds, in doses of 60 c. c. in capsules, failed to destroy one *Habronema muscae* in one animal; was 55.5 per cent effective for this species in a second animal; and was 97.2 per cent effective for *H. microstoma* in a third animal. It seems probable that a preliminary gastric lavage would add to the efficacy of n-butylidene chloride against *Habronema* spp.

The drug was relatively ineffective against bots. Species of parasites other than those mentioned above were not present in sufficient numbers to warrant final conclusions.

N-butylidene chloride was well tolerated and produced no gross pathological alterations. Microscopical pathology in the livers of treated animals was confined to cloudy swelling with some fatty degeneration but no central necrosis. In two of three animals, the drug produced only a hyperemia of the kidneys, but in the third animal cloudy swelling and degeneration of the tubular epithelium were present. The severity of the lesions in this animal was apparently associated with increased absorption of the anthelmintic due to insufficient purgation.

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HIGH AND LOW LETHAL TEMPERATURES FOR THE WESTERN PINE BEETLE[1]

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INTRODUCTION

The fact that wood borers and bark beetles may be killed as a result of the heat absorbed by the dead host plant when exposed to sunlight has been known for a number of years. The first published observations of solar heat as lethal to forest insects were made in 1917 by Craighead (*2*),[2] who found that many pupae and immature adults of a cerambycid borer, *Cyllene pictus* Drury, were killed when infested logs were exposed to the sun. In 1919 and 1920 experiments carried out by J. E. Patterson and the writer in Oregon and California proved the effectiveness of solar heat in producing killing temperatures in yellow-pine bark infested by broods of the western pine beetle (*Dendroctonus brevicomis* Lec.). It was found that complete mortality resulted when bark temperatures reached 120° F., and that bark removed from the tree and placed in sunlight reached this and even higher points when the air temperatures in the shade ranged from 85° to 90°.

On the other hand, extreme cold may likewise result in the killing of bark-beetle broods. For example, in December, 1924, unusually low minimum temperatures of from −20° to −25° F. were recorded in the vicinity of Bend, Oreg. After this cold spell, A. J. Jaenicke, of the Forest Service, United States Department of Agriculture, found that a high percentage of the overwintering larvae were dead through a considerable area of yellow pine infested by the western pine beetle. This mortality, which ranged from 25 to 80 per cent, could be accounted for only as a result of freezing of the larvae in the outer bark.

In the winter of 1926–27, at Asheville, N. C., Beal (*1*) found that general and heavy mortality of the southern pine beetle (*Dendroctonus frontalis* Zimm.) occurred within a few days after the first severe cold in January. Hopkins (*3*) attributed the sudden decline of an epidemic of this beetle in West Virginia to the severe freeze in the winter of 1892–93, but later was of the opinion (*3, p. 376*) that disease was an important contributing factor.

A general treatment of the subject of weather and climate in their relation to insects has recently been published by Uvarov (*9*).

[1] Received for publication Feb. 26, 1931; issued September, 1931.
[2] Reference is made by number (italic) to Literature Cited, p. 321.

Other workers in this field have gone more minutely into the effects of low temperatures within the body of the insect itself. Robinson (*7*) has developed a method for determining the natural undercooling and freezing points in insects. This worker (*8*) has also shown the water-binding capacity of colloids to be a definite factor in the ability of insects to resist the cold of winter. Several papers by Payne (*4*, *5*, *6*) also deal with the factors involved in the resistance of insects to cold.

The effects of temperatures on the brood stages of the western pine beetle have been studied since 1920 under both field and laboratory conditions by the division of forest insects of the Bureau of Entomology. Laboratory experiments were conducted at Stanford University, Calif., and field studies were pursued at points in California, Oregon, and Idaho. The object of these studies was to determine (1) the high temperatures which result in mortality when solar heat is used for control and (2) the low temperatures which result in the death of the overwintering brood stages when severe cold weather occurs in infested areas.

The results of these studies, as far as they had progressed at the end of 1929, are presented in this paper.

MATERIAL SELECTED FOR STUDY

The material which has been used for testing the effect of high and low temperatures consisted mainly of sections of yellow pine bark infested by the various brood stages of the western pine beetle. The term "brood" is used to designate the progeny of a pair of parent beetles during the period of development within the tree. As used throughout this paper, this term applies to eggs, larvae, pupae, and new adults, as they are found collectively in the bark samples.

Trees are attacked during the active period, late in the spring, in summer, and early in the fall, by the parent adults, which make winding tunnels through the live cambium and deposit eggs in notches cut along the sides of these tunnels. After the eggs hatch the young larvae feed for a time in the cambium and inner bark, then bore directly into the corky layers of the outer bark, where they complete their development. Each larva cuts in the outer bark the individual feeding gallery and the cell in which it transforms, first to pupa, then to new adult. Consequently any temperatures affecting the larvae and later stages must reach the insect through the protective covering of the outer bark. The cambium and bark contain the primary infestation from the time the tree is attacked until it is eventually abandoned by the new adult beetles. However, the bark is the part of the tree in which the broods will be found during both the midsummer and the midwinter, when lethal temperatures occur.

In determining the effects of both high and low temperatures, the temperature of the outer bark, as recorded by embedded thermometers (fig. 1), has been used as a basis for correlation with mortality of broods. Records have also been obtained of the effect of air temperatures on larvae, pupae, and adults exposed in open containers after removal from the bark.

MORTALITY RESULTING FROM EXPOSURE OF INFESTED BARK TO SOLAR HEAT

In experiments with solar heat as a lethal agent there is no means of controlling the degree of heat to which the bark is exposed, as this is dependent entirely upon weather conditions. It is obvious that the only course open to the experimenter is to record the temperatures

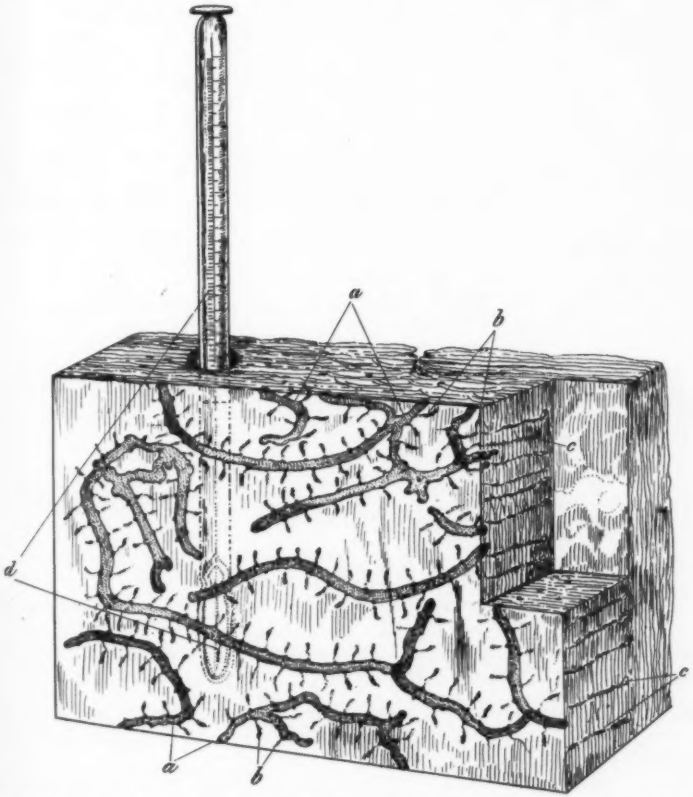

FIGURE 1.—Section of infested bark of the western yellow pine, showing position of thermometer used in recording temperatures: *a*, Egg galleries of western pine beetle on inner surface of bark next the wood; *b*, mines of young larvae in inner bark; *c*, mines of older larvae and pupal cells in outer bark; *d*, mercurial thermometer with bulb embedded at depth of pupal cells and calibrated scale extending beyond bark

developed within the bark and compare them with the resultant mortality of the broods. However, the quantity of heat that is absorbed by the bark varies decidedly with the angle at which the bark is inclined toward the sun. The maximum is reached in the middle of the day, provided the bark is placed as nearly as possible with its surface at an angle of 90° to the sun's rays. On a standing tree the bark surface of the main trunk is vertical, and therefore nearly par-

allel to the direction of the sun's rays at midday during the summer season. This position, together with the contact of the bark on the sapwood, results in conditions which keep the bark temperatures well below those producing mortality, even though infested bark on the lower part of the trunk may be exposed to the sun at midday.

This point is illustrated by Figure 2, which is a chart graphically presenting records taken under forest conditions near Ashland, Oreg., February 11, 1920. An infested tree was selected and thermometers were placed about it as follows: One weather thermometer suspended in the shade, about 10 feet from the tree, to record air temperature; one mercurial stirring-rod thermometer embedded in infested bark on the north side of the tree, 5 feet above the base; one mercurial stirring-rod thermometer embedded in infested bark on the south side of the tree, 5 feet above the base; and one mercurial stirring-rod thermometer embedded in a section of infested bark measuring 12 by 24 inches which had been removed from the tree and

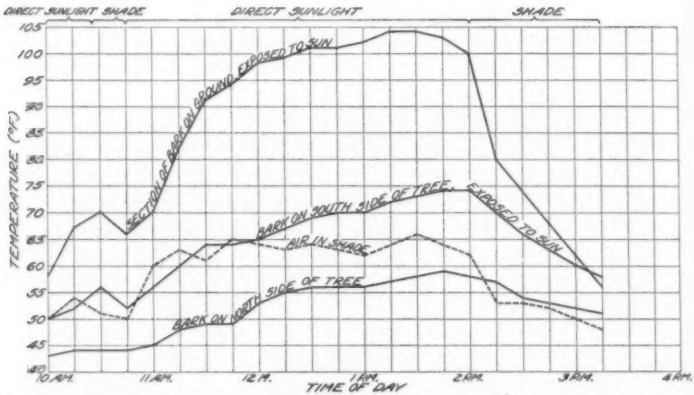

FIGURE 2.—Comparison of temperatures of bark of the western yellow pine, infested by the western pine beetle, under different conditions of exposure to solar heat, and of the near-by air, in the shade, February 11, 1920

placed on the ground at its base. The inner surface of the bark was turned upward, in a position approximately at an angle of 90° to the sun's rays at midday.

Readings from all four thermometers were taken at 15-minute intervals from 10 a. m. until 3.15 p. m. The entire day remained clear, but shadows from near-by trees caused periods of shade from about 10.25 to 10.45 a. m. and from 2 p. m. until sundown.

The readings show that the temperature of the bark placed on the ground climbed rapidly when exposed to the sun until 1.30 p. m., when it reached a maximum of 104° F., or 38 degrees higher than the corresponding air temperature. After 1.30 p. m. the temperature of this bark declined slowly until 2 p. m., when the shade came on, and it fell rapidly during the next hour.

The bark exposed to the sun on the south side of the tree did not become much warmer than the air in the shade until 12 m. Its temperature reached a maximum of 74° F. at 2 p. m., or 8 degrees

higher than the air temperature at 1.30 p. m. This bark did not begin to cool until 2 p. m.

The temperature of the bark on the north side of the tree lagged well below the temperature of the air and of the bark on the south side throughout the period of sunlight. After 2 p. m., as the temperature of the air became lower, the bark attached to the tree retained its heat, and its temperature on both the north and the south sides was higher than the air temperature at 3.15 p. m.

This experiment indicates that infested bark attached to a standing tree does not become more than 10° warmer than the corresponding air temperature in the shade, and that it is necessary to remove infested bark and expose it flat on the ground in order that it may absorb sufficient heat to insure higher temperatures.

During the summer of 1920, experiments were conducted to determine what bark temperatures are necessary to produce effective mortality of the western pine beetle and the conditions which are essential in order to produce these lethal temperatures by solar heat. Three tests were carried out by J. E. Patterson, at Pinehurst, in southern Oregon, while five tests were made by the writer at North Fork, in central California. The months of June, July, and August were selected for these tests, as this is the period when, because of fire hazard, it is desirable to treat the summer broods by the application of solar heat rather than by burning.

The method used in running these tests was as follows:

(1) A series of from 10 to 20 sections of infested bark, measuring 12 by 24 inches, were taken from the trees early in the morning and spread upon the ground where they would be in sunlight from 10 a. m. to 3 p. m.

(2) Thermometers were embedded in two of the sections, and readings were taken at intervals of 15 or 30 minutes throughout the period of exposure.

(3) A thermograph was set up within a few yards of the place where the bark was exposed, which recorded at the same intervals the air temperature in the shade.

(4) At each interval when temperatures were recorded a section of bark was taken from the series exposed on the ground, and placed in the shade, where it remained under ordinary air temperatures until broken up in order that live and dead individuals of the broods might be counted.

(5) Mortality was determined several days after exposure by breaking and shaving the sections so as to expose the broods and count the number of live and dead individuals.

Table 1 is a summary of one of these tests at North Fork, Calif., June 23, 1920. Temperatures of the two sections in which thermometers were embedded are shown, together with the corresponding air temperatures in the shade. The mortality found afterward in the sections removed at the time readings were taken is also shown. It should be noted that the two sections in which thermometers were embedded did not become warm or cool at the same rate, nor did they reach the same maximum. However, their temperatures were at all times within 8 degrees of each other. The temperature of the section removed at a time of reading may have differed to this extent from those in which thermometers were placed. These differences

can be explained as due to varying thicknesses of bark, slight differences in tilt or angle of exposure, and varying movements of air around the sections. It is altogether probable that the temperature of the section removed at 10 a. m., in which a mortality of 34.2 per cent was found, exceeded by a few degrees the temperatures of 99° and 100° F. recorded by the embedded thermometers in other sections. Uniformity of temperatures in the sections could not be expected under the conditions of the experiment, the main purpose being to determine under what air temperatures the bark would absorb enough heat to compensate for all these variations, so as to assure killing of a high percentage of the broods. The outstanding points shown by this test are that, with air temperature around 80°, the infested bark by 11.30 a. m. had absorbed enough heat to make its temperature exceed 120°, and that all sections removed after this time showed a mortality of 100 per cent. The maximum spread between bark and air temperatures was observed at 1.30 p. m., when the bark was approximately 50 degrees warmer than the air.

TABLE 1.—*Comparison of temperatures in sections of infested bark exposed to direct sunlight, coincident air temperatures, and subsequent mortality of broods of the western pine beetle, North Fork, Calif., June 23, 1920*

Time	Air temperature in shade	Relative atmospheric humidity	Temperature recorded in bark by embedded thermometer		Condition of larvae and pupae in bark after removal from sunlight (examined July 7, 1920)		
			Section No. 1	Section No. 2	Alive	Dead	Mortality
	° *F.*	*Per cent*	° *F.*	° *F.*	*Number*	*Number*	*Per cent*
9.30 a. m.	78	25	76	76	150	2	1.3
10 a. m.	80	25	99	100	96	50	34.2
10.30 a. m.	79	28	117	109	31	114	78.6
11 a. m.	80	28	122	118	11	186	94.4
11.30 a. m.	79	28	122	120	0	186	100
12 m.	80	28	124	124	0	185	100
12.30 p. m.	81	25	128	128	0	153	100
12.55 p. m.	81	25	128	128	0	239	100
1.30 p. m.	81	20	132	128	0	227	100
2.10 p. m.	84	14	132	126	0	250	100
3 p. m.	83	12	128	122	0	309	100

The conditions of other tests were varied as to weather conditions and as to the time of day, duration of exposure, and angle of exposure of bark to the sun's rays. In general, it was found that bark removed from the log and placed on the ground at an angle of from 60° to 90° to the sun's rays in the middle of the day acquired temperatures of from 110° to 132° F., when corresponding air temperatures in the shade ranged from 80° to 95°.

The results of these studies warrant the following general conclusions:

(1) No mortality due to heat results after brief exposures if the acquired temperatures of the bark do not exceed 100° F.

(2) A mortality of 40 to 90 per cent results after several days of exposure when the temperatures of the bark reach maxima ranging from 100° to 115° F.

(3) A mortality of 100 per cent results from an exposure of two or three hours when the temperatures of the bark reach a maximum of 115° to 118° F.

(4) A mortality of 100 per cent results from exposures of approximately one hour when the temperatures of the bark reach 120° or higher.

In these tests the brood stages range from half-grown to full-grown larvae, with a small percentage of pupae and new adults. No attempt was made to separate the resistance of different stages of brood to high temperatures, but it was apparent that pupae and adults are killed by the same high temperatures that are fatal to the larvae.

In these tests with solar heat the factor of relative atmospheric humidity was not isolated from that of temperature. The amount of moisture in the bark is apparently the determining factor in the humidity of the environment of advanced stages of brood. After bark is removed from a tree and exposed to the sun a drying-out process takes place, and as the amount of moisture decreases the rate at which the bark warms up to high temperatures may be accelerated. This angle of the matter has not been studied, but in the general practice of the method of control by solar heat it has been found that after two or three days the killing of the broods is more effective than during the first day of exposure.

MORTALITY OF LARVAE WHEN EXPOSED TO HIGH TEMPERATURES OF THE AIR

As it is impossible to observe the behavior of larvae or other brood stages when they are exposed in the bark to critical temperatures, a series of laboratory tests was carried out with half-grown to full-grown larvae removed from the bark and exposed to varied temperatures of the air. In one test, conditions permitting free evaporation were provided by exposing the larvae to currents of warm air in circulation. As a contrast, conditions in which evaporation was reduced to a minimum were provided by sealing the larvae in glass vials and submerging these under water, which was then slowly heated through the range of critical high temperatures. The results of the tests are shown in Table 2.

TABLE 2.—*Effect of high temperatures of the air on larvae of the western pine beetle removed from bark of the western yellow pine, February, 1923*

Temperature	Lot 1. Effect on 100 larvae in 5 groups of 20 each in open pasteboard containers [a]	Lot 2. Effect on 100 larvae in 5 groups of 20 each in glass vials sealed with paraffin [b]
°F.		
70 to 90	Normally active	Normally active.
90 to 95	Maximum activity	Maximum activity.
95 to 100	Slight activity; paralysis after prolonged exposure.	Activity decreases.
100 to 105	Paralysis after brief exposure; complete mortality after 30 minutes' exposure at 105°.	Slight activity continues up to 105°.
105 to 110	Fatal after brief exposure	Paralysis of larvae occurs after long exposure (several hours) at 108° to 110°; larvae recover if returned to normal temperatures.
110 to 115	do	Paralysis occurs after 20 minutes' exposure; larvae do not recover if returned to normal temperatures.
115 to 120	do	Complete mortality occurs after long exposure between 115° and 118°; no larvae survive even brief exposure above 118°.

[a] These were exposed at different heights over an electric plate, permitting free circulation of air and evaporation.

[b] These vials were submerged in water which was slowly heated through the range of temperatures indicated.

Larvae in the sealed containers were killed within the same approximate range of temperatures as those in the outer bark. However, in the open-air containers which permitted free evaporation the critical temperatures were 5 to 8 degrees lower and mortality was practically complete at 110° F.

The condition termed paralysis was also observed in certain larvae removed from the bark after test with solar heat. When it occurs all movement of the insect ceases, and the insect fails to respond to a stimulus such as the touch of a camel's-hair brush. The larvae do not, however, take on the discolored appearance which develops within a day or so after they have been killed. Paralyzed larvae may recover and continue their development if the temperatures producing this condition are not too prolonged. In lot 2 some of the larvae that were paralyzed at 115° remained in this dormant condition for several months and then died.

CHARACTER OF FREEZING EXPERIMENTS AND EQUIPMENT

During the winters of 1926–27, 1927–28, and 1928–29 a series of tests was carried out by members of the field laboratory of the Bureau of Entomology at Stanford University, Calif., to determine the freezing temperatures that are fatal to the western pine beetle. As in the tests with solar heat, the temperatures of the bark were indicated by embedded mercurial thermometers. The apparatus used to determine these temperatures at the depth of the infesting broods is shown in Figure 1. The entire calibrated scale used on the glass-rod thermometer extends outside the bark, so that it is possible to take readings without disturbing the bulb.

Low temperatures were provided and controlled by the use of specially designed apparatus. In an insulated tank of brine were two compartments 10 by 16 by 24 inches in size, used as containers of infested bark, and covered by insulated lids. In the brine was a coil in which the vapor of carbon disulphide was compressed by mechanism operated by a small electric motor. Temperatures of the bark and the surrounding air were read when the lids were removed, the operation requiring less than 30 seconds. This arrangement obviated the use of thermocouples, which would have been of little advantage, since in nearly all experiments it was necessary to open the cabinet at each reading in order to take out a section of infested bark. Owing to the thickness of the bark little change in its temperature occurred during the brief interval while readings were taken.

Sections of infested bark of about 2 square feet each were used as units in these tests. Each test, which included a group of 6 to 10 sections, was started with temperatures in the compartments slightly below that of the laboratory. The temperatures were then lowered until those of the bark had reached the desired minimum. At prescribed intervals sections were removed from the compartment and kept in the laboratory under normal room temperatures. After 24 hours or more the bark was broken up, the brood removed, and a count made of live and dead individuals. In doubtful cases the brood was kept under observation for several days, or even longer, until dead individuals could be distinguished by discoloration from dormant ones which were recovering.

Figure 3 is a photograph showing live and dead larvae removed from bark after exposure to a temperature of −5° F. Larvae killed by freezing have a characteristic blackened appearance which readily distinguishes them from those which survive.

The material used was obtained from two regional areas representing different climatic conditions. The greater part came from infested trees near North Fork, Calif., where winters are mild and minimum temperatures seldom fall below 10° F. A smaller quantity of material was obtained from Coeur d'Alene, Idaho, where winter temperatures occasionally fall as low as −20°.

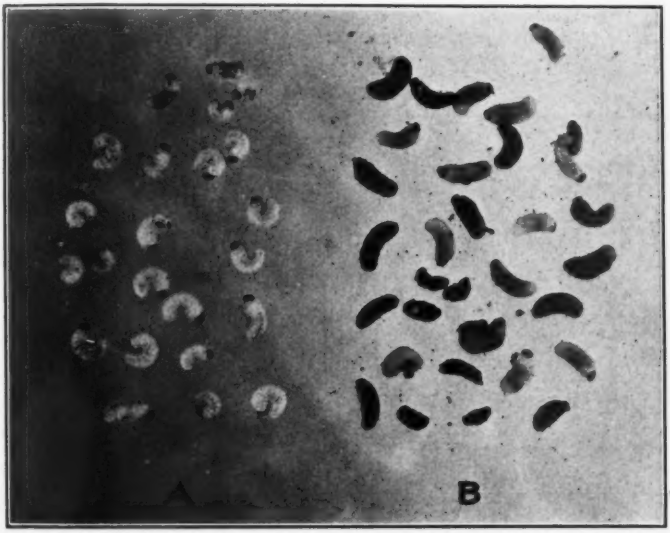

FIGURE 3.—Larvae of western pine beetle removed from bark two days after exposure to a temperature of −5° F.: A, 23 living larvae which survived; these are normal in color and active when disturbed; when not in motion they usually lie curled, with head and anal end turned toward ventral surface of body. B, 32 dead larvae; these are discolored and show no activity; the tissues of the body are soft, and the larvae usually lie considerably distended

EFFECTS OF LOW TEMPERATURES ON LARVAE UNDER VARYING CONDITIONS OF EXPOSURE

The method of exposure to low temperatures in the freezing cabinet was varied (1) as to the time in which bark was cooled from normal to subzero temperatures, and (2) as to the condition of the brood—whether dormant or active. The time during which the bark was allowed to warm back from subzero to normal temperatures was also varied, but it was soon found that the time required for return to normal temperatures did not affect the resultant mortality after fatal low temperatures had once been reached.

The results obtained from five differently treated groups of sections of bark from the material collected at North Fork are summarized in Table 3. In some of this material a mortality of 1 to 3 per cent of the larvae in advanced stages of development had already

occurred in the field at the time the bark was collected, owing in part to parasites and to other causes not determined. However, the low mortality from natural causes did not materially affect the results of freezing. Mortality considerably in excess of that found under normal conditions resulted, even at the first critical temperatures between 5° and 0° F.

Results shown in Table 3 indicate that, regardless of the variations introduced by differences in rate of cooling and in condition of the broods, mortality of the larvae due to cold appears between 5° and 0° F.; mortality exceeds 57 per cent at 5°, and is practically complete at −10° F.

TABLE 3.—*Effects of low temperatures on larvae of the western pine beetle under varying conditions of exposure, 1928*

Conditions of experiment	Bark section No.	Removed from cabinet: Date	Removed from cabinet: Hour	Temperature of bark when removed	Date of examination	Larvae: Alive	Larvae: Dead	Mortality
				° F.		Number	Number	Per cent
LOT 1								
Bark transferred from field and placed in cabinet after short period in laboratory; temperature lowered from 45° to −18° within 48 hours	Check.	Jan. 4	11.20 a. m.	46	Jan. 7	185	0	0
	1	do	2.15 p. m.	25	Jan. 5	23	0	0
	2	do	3.25 p. m.	20	do	11	0	0
	3	do	4.30 p. m.	15	do	43	0	0
	4	do	6 p. m.	10	Jan. 6	135	0	0
	5	do	7.45 p. m.	5	do	27	0	0
	6	do	9.45 p. m.	0	do	148	33	18.2
	7	Jan. 5	10.45 a. m.	−5	Jan. 6, 18	53	150	73.9
	8	do	12.45 p. m.	−10	do	7	248	97.3
	9	do	4.25 p. m.	−15	Jan. 6, 19	0	168	100.0
	10	Jan. 6	8.40 a. m.	−18	do	0	181	100.0
	11	Jan. 8	4 p. m.	+26	Jan. 19	0	97	100.0
LOT 2								
Bark transferred from field and placed in cabinet after short period in laboratory; temperature lowered gradually from 45° to −15° within 216 hours	Check.	Jan. 11	10.30 a. m.	48	Jan. 13	100	0	0
	1	Jan. 12	12.30 p. m.	20	do	100	0	0
	2	Jan. 13	4.25 p. m.	15	Jan. 20	103	0	0
	3	Jan. 14	4.30 p. m.	10	do	85	0	0
	4	Jan. 15	2.45 p. m.	5	Jan. 21	165	0	0
	5	Jan. 17	4.30 p. m.	−1	Jan. 18	103	23	18.3
	6	Jan. 18	2.15 p. m.	−5	Jan. 19	34	133	79.6
	7	Jan. 19	8.30 p. m.	−14	Jan. 20	0	153	100.0
	8	Jan. 25	5 p. m.	+39	Jan. 27	0	203	100.0
LOT 3								
Bark transferred directly from field to cabinet with broods in dormant overwintering condition; temperature lowered from 48° to −15° in 48 hours	Check.	Jan. 18	9 a. m.	48	Jan. 18	114	4	3.4
	1	do	2.15 p. m.	19	Jan. 19	97	3	3.0
	2	do	3.20 p. m.	14	do	99	3	2.9
	3	do	4.20 p. m.	10	Jan. 20	97	3	3.0
	4	do	5.20 p. m.	6	do	98	2	2.0
	5	do	6.15 p. m.	−3	do	71	32	31.1
	6	do	9 p. m.	−5	do	11	17	60.7
	7	Jan. 19	2 p. m.	−10	do	0	60	100.0
	8	do	7.45 p. m.	−15	do	0	80	100.0
	9	Jan. 20	6 p. m.	−20	Jan. 21	0	98	100.0

TABLE 3.—*Effects of low temperatures on larvae of the western pine beetle under varying conditions of exposure, 1928*—Continued

LOT 4

Conditions of experiment	Bark section No.	Removed from cabinet: Date	Removed from cabinet: Hour	Temperature of bark when removed	Date of examination	Larvae: Alive	Larvae: Dead	Mortality
				° F		Number	Number	Per cent
Bark transferred from field to laboratory and kept at 60°–70° for 21 days, then transferred to freezing cabinet with larvae active and developing; temperature lowered from 60° to −15° in 48 hours.	Check	Feb. 7	10. a. m.	60	Feb. 7	220	4	1.8
	1	do	4.50 p. m.	20	Feb. 8	135	6	4.3
	2	do	6.45 p. m.	15	do	140	4	2.8
	3	do	8.10 p. m.	10	do	143	5	3.4
	4	Feb. 8	8.50 a. m.	4	Feb. 10	125	3	2.3
	5	do	10.20 a. m.	0	do	26	4	13.3
	6	do	2 p. m.	−5	do	70	93	57.1
	7	do	4.30 p. m.	−7	do	0	15	100.0
	8	do	9 p. m.	−10	do	0	70	100.0

LOT 5

Bark transferred from field to laboratory and subjected to daily changes of temperature ranging from 25° to 70° for six days, then transferred to cabinet and temperature lowered from 70° to −15° within 48 hours; preliminary variations of temperature continued from Feb. 14 to Feb. 20.	Check[a]	Feb. 16	3.30 p. m.	26	Feb. 16	340	7	2.0
	1	Feb. 20	11.30 a. m.	25	Feb. 23	126	2	1.6
	2	do	1.45 p. m.	20	do	100	1	1.0
	3	do	2.30 p. m.	15	do	98	2	2.0
	4	do	4.30 p. m.	10	do	105	0	0
	5	do	8.15 p. m.	5	do	110	2	1.8
	6	Feb. 21	1.30 p. m.	0	do	88	17	16.2
	7	do	4. p. m.	−5	do	46	75	62.0
	8	do	5.30 p. m.	−7	do	22	120	84.5
	9	do	8.30 p. m.	−9	do	0	100	100.0
	10	do	9.40 p. m.	−11	do	0	100	100.0

[a] This check removed on third day, after 3 days' exposure to temperatures ranging from 26° to 74° F.

In lot 5 an effort was made to simulate the daily range of temperatures in the field, where cold nights and warm days are a feature of fall weather when the broods are preparing for the overwintering period. In planning this test, consideration was given to the possibility that short periods of cold, producing dormancy, with intervening periods permitting activity, might enable the broods to undergo a hardening process and withstand much lower temperatures than if suddenly exposed to critical temperatures.

This assumption was supported in the case of other experiments with broods of the mountain pine beetle (*Dendroctonus monticolae* Hopk.). After the larvae had been subjected to temperatures fluctuating from 20° to 50° F. for a period of several days, they were observed to discharge a considerable amount of excrement from the alimentary canal. After going through this process their resistance to low temperatures increased, and a high percentage of the broods survived temperatures of −15° F.

However, no tendency was observed on the part of the larvae of the western pine beetle to empty the alimentary canal, and their resistance to freezing was apparently not increased by any variations of temperature preceding exposure to the fatal freezing points. In fact, lot 5 showed a perceptible mortality somewhat higher at 5° F. than was found in the other material which was carried to subzero temperatures without preliminary fluctuations permitting short periods of activity.

EFFECTS OF LOW TEMPERATURES UPON PUPAE AND NEW ADULTS

Some of the material collected from North Fork was kept under laboratory temperatures until the greater part of the broods had developed to the pupal stage, and then lowered through the same range of temperatures as that containing larvae in the preceding tests. A similar test was made of another lot of material after the broods had just reached the adult stage and were resting in the outer bark. The results from this material are summarized in Table 4.

TABLE 4.—*Mortality of pupae and new adults of the western pine beetle resulting from low temperatures of bark infested by them, 1928*

PUPAE [a]

Bark section No.	Removed from cabinet: Date	Removed from cabinet: Hour	Temperature of bark when removed	Date of examination	Alive	Dead	Mortality
	1928		°F.	1928	*Number*	*Number*	*Per cent*
1	Mar. 1	3 p. m.	26	Mar. 5	94	0	0
2	do	4.30 p. m.	21	do	80	0	0
3	do	7.15 p. m.	15	do	86	0	0
4	do	9 p. m.	11	do	84	0	0
5	Mar. 2	8 a. m.	6	do	84	16	16.0
6	do	10 a. m.	0	Mar. 6	75	27	26.5
7	do	11.30 a. m.	−5	do	1	99	99.0
8	do	3 p. m.	−8	do	0	122	100.0
9	do	4 p. m.	−10	do	0	120	100.0

NEW ADULTS [b]

1	Mar. 13	3.45 p. m.	20	Mar. 20	90	0	0
2	do	7 p. m.	15	do	82	10	10.9
3	do	9 p. m.	10	do	7	70	90.9
4	Mar. 14	7 a. m.	5	do	2	73	97.3
5	do	10 a. m.	0	do	3	66	95.7
6	do	1 p. m.	−5	do	0	48	100.0
7	do	2.45 p. m.	−10	do	0	68	100.0

[a] The infested bark was collected at North Fork, Calif., in December, 1927. It was kept in the laboratory 1 month before this test was begun, about 80 per cent of the brood having meanwhile transformed to pupae.
[b] The infested bark was kept under laboratory temperatures until the majority of the brood had transformed to adults and were ready to emerge.

These results indicate that pupae are somewhat less resistant to cold than larvae, as mortality was practically complete at −5° F. Adults, however, appear to be decidedly more susceptible than either larvae or pupae. With them a high mortality results from exposure at 10°, and mortality is complete at −5° F.

EFFECTS OF LOW TEMPERATURES UPON EGGS IN THE CAMBIUM

Difficulties not encountered with broods in the advanced stages in the outer bark were found in obtaining records of temperatures destructive to the eggs. The eggs are found only in notches excavated by the parent in the cambium, and removal of the bark from the sapwood disturbs the natural conditions for incubation. Mortality due to freezing, therefore, can not be isolated from other factors unfavorable to incubation and development of the eggs.

Freshly attacked logs about 8 inches in diameter, in which parent adults were depositing eggs, were selected for these tests. These were cut into blocks about 6 inches in length and subjected in the freezing cabinet to a range of temperatures similar to those used with the other brood stages. Temperature records of the cambium layer were obtained by means of a thermometer with the bulb embedded at its depth under the bark. After exposure the blocks were allowed to stand under laboratory temperatures of 60° to 75° F. for about 10 days. The bark was then shaved from the sapwood so as to uncover the egg galleries, and a count was made of eggs which had developed and of those which had failed to develop. (Table 5.) A characteristic bluish color distinguished the eggs that had apparently been killed as a result of freezing.

TABLE 5.—*Destruction of eggs of the western pine beetle resulting from low temperatures in the cambium in which they had been deposited*

Bark section No.	Removed from cabinet		Temperature of cambium when removed	Date of subsequent examination	Eggs developed	Eggs failing to develop	
	Date	Hour					
	1928		°F.	1928	*Number*	*Number*	*Per cent*
1	May 23		50	June 2	169	9	5.1
2	May 24	9.30 a. m.	15	do	54	3	5.3
3	do	12 m.	10	do	48	9	15.8
4	do	2.17 p. m.	5	do	45	26	36.6
5	do	4.30 p. m.	0	do	51	55	51.9
6	May 25	2.35 p. m.	−5	June 6	22	22	50.0
7	May 26	9.45 a. m.	−10	do	9	88	90.7
8	May 27	7.30 a. m.	−15	do	11	76	87.4

It was found that the temperatures of the cambium layer within the blocks varied considerably with the moisture content, and that they did not decrease uniformly, as was the case with infested bark removed from the log. Because of this factor and the limited quantity of material available, these counts do not furnish a satisfactory basis for determining the rate of destruction; however, the fact that at least 10 per cent of the eggs developed after they had been subjected to temperatures around −10° F. indicates that eggs are more resistant to cold than are larvae, pupae, or adults.

EFFECTS OF VARIOUS TEMPERATURES ON LARVAE IN OUTER BARK SATURATED WITH WATER

Studies of control by the method of submerging infested logs in water at temperatures of 50° to 65° F., carried on during the season of 1929, brought out the fact that larvae soon become dormant under water and remain in this condition for approximately four weeks before mortality occurs. If removed from the water and allowed to dry before this period expires, the larvae resume development. In order to determine whether they lose their resistance to cold in the condition of dormancy produced by saturation, infested bark was soaked in water for seven days and then subjected to low temperatures. A check consisting of dry bark from the same material was run through a similar range of temperatures. The mortality at critical temperatures is shown in Table 6.

TABLE 6.—*Mortality of larvae of the western pine beetle in saturated bark compared with mortality in dry bark when exposed to low temperatures*

DRY BARK

Bark section No.	Removed from cabinet		Temperature when removed	Date of examination	Larvae		Mortality
	Date	Hour			Alive	Dead	
	1929		°F.	1929	*Number*	*Number*	*Per cent*
Check.	Jan. 14	1 p. m.	50	Jan. 14	100	2	2.0
1	Jan. 28	1 p. m.	15	Jan. 30	100	3	2.9
2	do	3 p. m.	8	do	98	2	2.0
3	Jan. 29	2.30 p. m.	5	Jan. 31	90	10	10.0
4	do	4 p. m.	−1	do	85	15	15.0
5	Jan. 30	2.30 p. m.	−4	Feb. 3	55	45	45.0
6	Jan. 31	5.05 p. m.	−8	do	0	100	100.0

SATURATED BARK

Check.	Feb. 25	9 a. m.	50	Feb. 25	112	2	1.8
1	Feb. 26	8 a. m.	24	Feb. 28	117	1	.8
2	do	10.30 a. m.	20	do	104	11	9.6
3	do	12 m.	15	do	96	6	5.9
4	do	1.30 p. m.	10	do	81	22	21.4
5	do	2.30 p. m.	5	do	60	46	43.4
6	do	4.15 p. m.	0	do	37	98	72.6
7	Feb. 27	2.45 p. m.	−5	do	5	100	95.2
8	do	4.15 p. m.	−8	do	0	100	100.0

This comparison indicates a perceptible lowering of resistance to freezing as a result of saturation of the bark. This degree of saturation would occur only under special conditions, such as that of logs stored in mill ponds. It is obvious that the degree of saturation necessary to cause dormancy rarely if ever occurs in standing infested trees under ordinary exposure to weather.

EFFECTS OF LOW TEMPERATURES OF THE AIR ON LARVAE REMOVED FROM OUTER BARK

In addition to tests with brood stages in the outer bark, a large number of larvae were exposed to varying temperatures in open pasteboard containers where their behavior could be observed. It was found that activity slowed down at about 50° F., and apparent dormancy set in at between 45° and 40°. Very little change could be observed at temperatures below 40° until temperatures between 15° and 10° were reached. At this point the body of the larva becomes rigid and takes on a peculiar white color. This change indicates the freezing point, where the free water in the body of the insect crystallizes. If larvae are brought back from this point to temperatures of 45° or higher, normal appearance and activity are resumed. However, if the temperature, instead of being raised, is lowered from the freezing point to subzero stages, mortality results. The critical temperatures established are shown in Table 7.

TABLE 7.—*Condition and mortality of half-grown to full-grown larvae of the western pine beetle removed from yellow pine bark and exposed to air temperatures ranging from 65° to −8° F., 1927* [a]

Lot No.	Removed from cabinet		Temperature of bark when removed	Condition of larvae when removed	Date of examination	Larvae		Mortality
	Date	Hour				Alive	Dead	
			° F.			*Number*	*Number*	*Per cent*
1	Feb. 11	9.10 a. m.	65	Active	Feb. 15	50	0	0
2	do	10.35 a. m.	45	Mostly active; few sluggish.	do	49	1	2
3	do	11.10 a. m.	40	Dormant	do	50	0	0
4	do	11.45 a. m.	35	do	do	50	0	0
5	do	3.15 p. m.	20	do	do	50	0	0
6	do	4.30 p. m.	15	do	do	50	0	0
7	do	5 p. m.	10	Dormant; frozen rigid	do	50	0	0
8	Feb. 12	12.45 p. m.	5	do	do	41	9	18
9	Feb. 15	1.05 p. m.	0	do	Feb. 18	41	9	18
10	do	3.30 p. m.	−5	do	do	21	29	58
11	do	5 p. m.	−8	do	do	0	50	100

[a] Material collected at North Fork, Calif., December, 1926; larvae removed from bark Feb. 10, 1927. Of these, 550 larvae were separated into 11 lots of 50 each and placed in open pasteboard containers. Temperature readings in freezing cabinet were taken with bulb of thermometer at same level as larvae in pasteboard containers.

Material was sent to William Robinson, at Chicago, who determined the freezing and undercooling points of individual larvae by special apparatus which he has devised. His records of 15 individual larvae show that the freezing point occurred at from 12° to 14° F., while the undercooling points where mortality resulted ranged from 8° to −2°. The general effect of both high and low temperatures and the results of tests of brood stages in the bark are shown in the following tabulation:

Temperature	Effect
° F.	
−20	Fatal to all brood stages.
−15, −10, −8	Fatal to all brood stages; a low percentage of eggs and of larvae which are adapted to severe winter climates may survive these temperatures.
−8, −5, 0, 5	Critical temperatures for larvae and pupae; mortality at −5° exceeds 50 per cent.
5, 10	Critical temperatures for adults; high mortality of adults occurs at 10°; low mortality of larvae and pupae may occur at 5°.
10, 15	Larvae freeze, but recover if warmed back to temperatures at which activity takes place.
15, 20, 35, 40	Dormancy occurs in all brood stages.
40, 45	Larvae sluggish.
45, 55	Larvae and adults active.
55, 95	Normal activity of larvae and adults.
95, 100	Larvae less active.
100, 105	Larvae sluggish.
105, 110	Paralysis of larvae after one or two hours' exposure; recovery possible if returned to lower temperatures.
110, 115	Paralysis of larvae after brief exposure; recovery possible if exposure is not prolonged.
115, 118	Paralysis of larvae after brief exposure (20 minutes), from which they do not recover.
120	Fatal to all brood stages.

EFFECTS OF LOW TEMPERATURES ON PARASITES AND PREDATORS IN THE BARK

Predators and parasites of the western pine beetle were found in many sections of infested bark used in the tests that have been described, and observations were made as to their mortality at various temperatures. However, this material was not sufficient in quantity to furnish a satisfactory basis for the determination of

critical temperatures, although the data secured indicate that these forms differ in their freezing points from the western pine beetle. In the case of an undetermined chalcid parasite the temperatures reached in these tests were not low enough to kill the larvae.

EFFECTS OF LOW TEMPERATURES IN REGIONS WHERE WINTER MINIMA FALL BELOW 0° F.

It is evident that in the northern limits of the distribution of the western pine beetle the winter temperatures fall below the critical points indicated by the tests described. Although weather records show that minimum temperatures as low as −20° F. may occur at times, at least part of the overwintering beetle population survives these cold spells. The survival may be due to a resistance which has been built up within the region by natural selection, or it may be due to the fact that temperatures in the bark of infested trees do not reach the low points recorded by weather thermometers in the surrounding air.

Through the cooperation of J. C. Evenden, of the field laboratory of the Bureau of Entomology at Coeur d'Alene, Idaho, where the winter climate is colder than that in the yellow-pine belt of the Sierra Nevada region, about 25 square feet of infested bark was mailed to the laboratory at Stanford University, Calif., and subjected to low temperatures in the freezing cabinet. This bark contained broods of larvae in advanced stages of development which had been in an overwintering condition for several months. During this period minimum temperatures as low as −10° F. had been recorded in the vicinity of the place where the material was collected. Bark sections from this material were cooled in the freezing cabinet from 55° to −18°, with the results shown in Table 8.

TABLE 8.—*Mortality of half-grown to full-grown larvae of the western pine beetle in yellow pine bark subjected to temperatures ranging from 55° to −18° F., 1927* [a]

Bark section No.	Removed from cabinet			Date of examination	Larvae		Mortality
	Date	Hour	Temperature		Alive	Dead	
			°F.		*Number*	*Number*	*Per cent*
1	Jan. 29	12 m	25	Feb. 2	112	0	0
[b] 2	...do	4 p. m	4	...do	61	12	16.4
3	...do	5 p. m	0	Jan. 31	119	0	0
4	...do	7 p. m	−5	Feb. 1	25	23	47.9
5	...do	8.50 p. m	−10	Jan. 31	25	81	76.4
6	...do	11 p. m	−12	Feb. 1	7	56	88.9
7	Feb. 1	8 a. m	−18	Feb. 2	11	207	93.0
8	...do	10.25 a. m	−18	...do	0	67	100.0

[a] Bark collected at Coeur d'Alene, Idaho, January, 1927, with broods in overwintering condition.
[b] The mortality found in section 2 can not be explained by temperatures to which the larvae were subjected in this test. It may have resulted from conditions in the field or in transit for which records are not available.

Although partial mortality appeared within the same range of critical temperatures as in the case of the material from North Fork, the striking difference is in the degree of survival after −5° F. had been reached. This amounted to a survival of 24 per cent at −10°, and of 11 per cent at −12°. Apparently these broods had developed

a partial resistance to temperatures which were 100 per cent fatal to broods that had overwintered under more favorable climatic conditions. However, this resistance was not sufficient to permit them to withstand temperatures more than 10 degrees below those which were fatal to the material collected at North Fork.

DIFFERENCE BETWEEN AIR AND BARK TEMPERATURES IN THE FIELD

The fact that broods of the western pine beetle do survive where weather records show minimum air temperatures below those which were found to be fatal in the bark indicates that the infested bark on standing trees does not become as cold as the surrounding atmosphere, and therefore does not cool down to the critical points. This seems apparent as the infested bark is in contact with the moist sapwood of the tree, which in turn connects with the root system in the soil. This position gives contact with a reserve of accumulated warmth which prevents rapid cooling of the bark. It would appear that only prolonged periods of subzero temperatures can chill the bark sufficiently to produce temperatures fatal to the broods.

Two series of records obtained by J. C. Evenden and H. J. Rust support this assumption. In January, 1927, and again in January, 1928, periods of subzero weather occured at Coeur d'Alene, Idaho. During these cold spells weather thermometers were placed in the bark on the north and south sides of an infested standing tree. At the same time thermometers were suspended in the air on the north, east, south, and west sides of the tree. Readings were taken hourly during a period of 28 hours. The lowest point recorded in the readings of air temperature was −6° F., when the corresponding bark temperatures were 4° and 6°. This shows a difference of 10° and 12° between the air and bark temperatures. As the air temperatures rose this difference decreased, and both air and bark temperatures corresponded closely at 12°. No appreciable mortality occurred in the broods; this result would be expected, since critical temperatures were not reached in the bark.

Further records of comparisons of air and bark temperatures and a study of the effects of extremely low temperatures in the field are necessary before it will be possible to determine the expectancy of mortality to broods of the western pine beetle as a result of winter freezing.

SUMMARY

Fatal temperatures are of importance in both the artificial and the natural control of the western pine beetle (*Dendroctonus brevicomis* Lec.). Temperatures in the bark that are high enough to result in mortality are essential in control by solar heat. Low temperatures in the field which result in the death of the broods from freezing may materially reduce the beetle population in infested areas.

High and low temperatures affecting brood stages of the western pine beetle while in the bark must reach the insects through the protective covering of the outer bark. Critical environmental temperatures must therefore occur in the outer bark.

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When infested bark is removed from the log and placed where it is exposed to sunlight during the midday period, it will reach temperatures ranging from 110° to 130° F. when the corresponding air temperature ranges from 80° to 95°. A partial mortality of the broods will result if bark temperatures range from 100° to 115° daily for a period of several days. Complete mortality is assured if bark temperatures reach 115° to 118° for a period of two hours or more. No broods survive a brief exposure if the temperature of the bark reaches a maximum of 120°.

When larvae are removed from the outer bark and exposed to warm air under conditions which retard evaporation, mortality occurs at about the same points of temperature as in the bark. If rapid evaporation is permitted, paralysis and mortality occur at temperatures about 5° lower than those at which they occur in the bark.

Mortality of the larvae follows freezing when bark temperatures are lowered below 0° F. Partial mortality occurs at zero in broods which overwinter in the yellow-pine belt of the Sierra Nevada region; mortality at −5° exceeds 60 per cent, and practically no larvae survive at −10°. Temperatures within the range from 0 to −10° are consistently critical, regardless of the rate at which the temperature of the bark is lowered or warmed back from subzero to normal temperatures; complete mortality develops within this range regardless of whether the larvae are actively developing or dormant at the time the bark temperature is lowered to the critical points.

High mortality of the pupae occurs at temperatures between 5° and −5° and is complete at −8° F.

High mortality of the adults occurs between 12° and 5° F., and is nearly complete at 0°.

Tests to determine the point of destruction of eggs were unsatisfactory and much less conclusive than the tests for other stages of development. Results available indicate that partial destruction of eggs in the cambium occurs at temperatures between 5° and −10° F. The destruction recorded at −15° was around 90 per cent, indicating that eggs have greater resistance to cold than do other brood stages.

When larvae become dormant through saturation of the bark with water they are less resistant to cold, and a mortality of about 20 per cent occurs at 10° F. Complete mortality, however, does not occur until points between −5° and −10° are reached.

When larvae are removed from the bark and exposed to cold air, complete mortality occurs at temperatures between 5° and −8° F.

The point at which the larvae freeze but recover if warmed back to active temperatures occurs between 10° and 15° F.

Larvae become dormant at about 40° to 45° F. and show no further change until the freezing point is reached.

Normal activity of the larvae occurs at temperatures between 55° and 90° F.

Broods which overwinter in regions where the minimum winter temperatures are below zero apparently develop greater resistance to cold than in regions with mild winter climates. Larvae which had passed through the overwintering condition at Coeur d'Alene, Idaho, where air temperatures of −10° F. had been reported, developed high mortality when bark temperatures were reduced through

the range from 0° to −10°. However, about 25 per cent survived a temperature of −10°. Mortality was practically complete at −18°. The difference between low temperatures withstood, owing to the influence of regional conditions, apparently does not exceed 10°.

The outer bark on standing infested trees cools down much more slowly than the surrounding air, and does not reach the minimum temperatures recorded by weather thermometers. Records obtained at Coeur d'Alene, Idaho, during two periods of subzero weather indicate that minimum bark temperatures are 10° to 12° higher than minimum air temperatures. Further records are needed to correlate this difference of temperatures between the bark and the air, in order to determine the probability of winter killing in infested areas.

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DEXIA VENTRALIS ALDRICH, AN IMPORTED PARASITE OF THE JAPANESE BEETLE [1]

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INTRODUCTION

Dexia ventralis Aldr. (fig. 1) was first found in Chosen (Korea) in the summer of 1922 by investigators from the Japanese beetle laboratory in search of parasites of the Japanese beetle (*Popillia japonica* Newm.). *D. ventralis* is fairly abundant over Chosen and the plains of Manchuria, but it is most abundant in central Chosen, where it has three complete generations on a variety of hosts, including *Miridiba koreana* N. and K., *Phyllopertha* sp., *Phyllophaga* spp., *Anomala* spp., *Serica* spp., and *Popillia* spp.[3] *P. japonica* does not occur in Chosen, but it was determined experimentally that Dexia would accept it as a host. In order to avoid the introduction of this parasite on any other host than *P. japonica* it became necessary to ship the parasite from Chosen to Japan and to secure the actual parasitizing of larvae of *P. japonica* in the latter country. All shipments mentioned, therefore, were made from Japan to the United States, the parasitizing of the Japanese beetle larvae having been brought about in Japan before shipment.

METHOD OF SHIPMENT

The time spent by *Dexia ventralis* in the pupal stage is only about two weeks, which makes it necessary to ship this parasite in the larval stage. Shipments are made in wooden trays about 18 inches square and 2 inches deep. These are divided by wooden cross-sections into 196 compartments, allowing a space about 1 by 1 by 2 inches for each compartment. Parasitized host grubs, distinguishable by the black spiracular sheath of the parasite larva which can easily be seen under the skin of the host, are secured and placed individually in these cells with sod and sterilized soil, and the cover is then nailed on the box. From three to five of these trays are placed in a larger wooden box for shipment. The wood of the box and trays retards evaporation and keeps the soil within the cells moist throughout the trip. During shipment the parasitized grubs feed on the sod.

The larval stage of *Dexia ventralis* is also very short except in the overwintering generation. When a shipment is received it is usual

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[2] The writer is greatly indebted to J. L. King, of the Japanese beetle project, for the many helpful suggestions and encouragement received during the course of this work.

[3] CLAUSEN, C. P., KING, J. L., and TERANISHI, C. THE PARASITES OF POPILLIA JAPONICA IN JAPAN AND CHOSEN (KOREA) AND THEIR INTRODUCTION INTO THE UNITED STATES. U. S. Dept. Agr. Bul. 1429, 56 p., illus. 1927.

to find that the parasite larvae have completed feeding, emerged from the host, and formed puparia while in transit. Occasionally an emerged adult is found.

This method of packing and shipping parasitized larvae of *Popillia japonica* makes a bulky container, but this is unavoidable. Soil-inhabiting, root-feeding forms can not very well be shipped without soil, and since the host has definite cannibalistic tendencies, individual isolation is necessary. Another drawback encountered in shipping is the loss of parasites sustained through mortality of the host. In

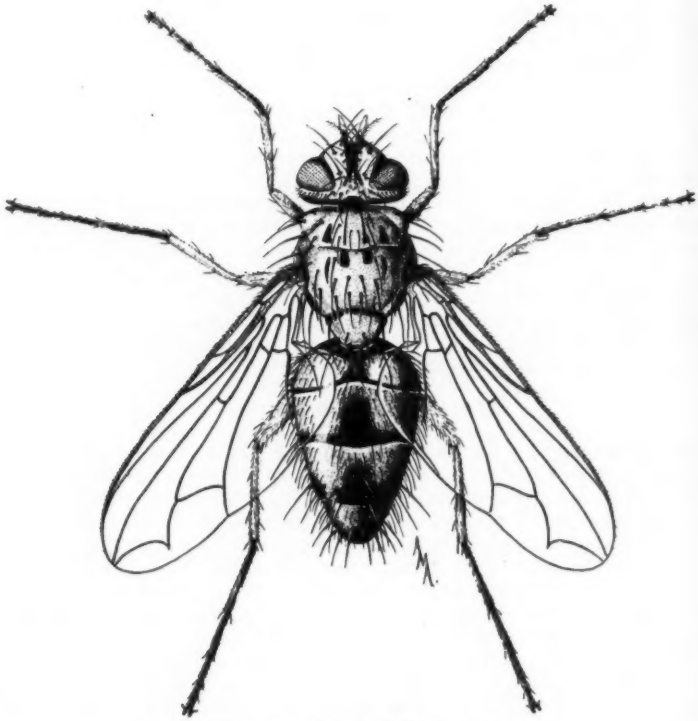

FIGURE 1.—*Dexia ventralis*, female adult. × 6½

the earlier shipments the ratio of adult parasites secured to host grubs shipped was less than 20 per cent, but improved shipping technic has raised this percentage to slightly higher than 30, and a single recent shipment had a percentage of nearly 50.

MANIPULATION AT THE INSECTARY

When a shipment of *Dexia ventralis* is received at the laboratory at Moorestown, N. J., the cases are opened in an insect-tight room, all contained soil is sifted, and the parasite material is sorted out. The shipping containers and the imported soil are then sterilized by

immersion in boiling water. The dexiid puparia and larvae obtained are placed in boxes on layers of moist sand, about 200 to each square foot. They are then covered with about an inch more of the sand and a layer of moist sphagnum. These boxes of puparia are put in large cloth cages, and the emerging flies are attracted to the walls, from which they are removed daily for colonization. In 1927 the emergence from puparia and larvae handled in this way averaged 85 per cent, and the emergence of a single shipment reached 98 per cent. The parasitized grubs from which the Dexia larvae have not yet emerged are repacked with fresh soil in trays similar to those used for shipping and placed in the cage to complete their development. For some reason parasitized hosts repacked in this way yield very few flies. In 1927 only 111 flies were secured from 1,693 hosts so repacked, or approximately 6 per cent.

COLONIZATION

Dexia ventralis is a crepuscular dexiid, and is moderately easy to manipulate for colonization, as it is not necessary to have mated females for colonization because of the gregarious habit of the species. After adults are released in a given locality, it is generally possible to observe them in the same area for several days. This habit is unusual, as many species of the closely related Tachinidae disperse immediately when liberated. Adult Dexia are found frequenting low shrub rows at the edge of grasslands. Ideal colonization sites are usually abundant on golf courses.

Four colonies of *Dexia ventralis* have been released in the field. The first was made on a golf course near Haddonfield, N. J., in 1926.[4] The next two were made in 1927, one on a golf course at Moorestown, N. J., and the other on Long Island, N. Y. These three colonies contained from 900 to 2,000 individuals of both sexes. The most recent colony, of 440 flies, was released in 1929 on a golf course in Pennsylvania, on a type of habitat different from those previously tried.

Of these colonies, recoveries have been made only from the one for which the flies were released in 1926. This colony consisted of approximately 1,500 flies, and since it was released in 1926, it must have gone through at least six complete generations in the field as indicated by life-history studies and scouting records.

RECOVERY

Recovery of this dexiid has been attempted in both the larval and adult stages. Several thousand overwintering host grubs were dug during the fall of 1927 and 1928, following a radial survey covering an area extending about a third of a mile in all directions from the center of colonization. The host grubs were all examined under the binocular microscope for evidence of parasitism, but no parasitism by Dexia was found. Parasitism is shown by the black larval spiracular sheath easily visible under the derm of the host. In the fall of 1929 no definite survey was made, but some 5,000 grubs were dug in connection with other work, and these yielded two adults of Dexia.

[4] KING, J. L., ALLEN H. W., AND HALLOCK, H. C. THE PRESENT STATUS OF THE WORK ON THE PARASITES OF POPILLIA JAPONICA NEWMAN. Jour. Econ. Ent. 20: 365–373. 1927.
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Recovery of this parasite in the adult stage has been successful and has been effected with less time and labor. Scouting is accomplished by careful observation of low-growing foliage at the edge of thickets during the morning or evening twilight periods.

The first recovery was made August 3, 1927, from liberations of the previous year. Seven specimens were taken at this time, some of which were sent to the United States National Museum for verification of the species determination. A summary of dates of recovery for three years is given in Table 1.

TABLE 1.—*Summary of recoveries of adults of Dexia ventralis at Moorestown, N. J.*

Year	Dates of recovery of adults from—				
	Brood 1		Brood 2		Brood 3 (?)
	First fly	Last fly	First fly	Last fly	
1927			Aug. 3	Aug. 26	
1928	June 16	June 29	Aug. 6	Aug. 16	Sept. 12
1929	June 11	June 26	July 30	Aug. 11	

It was possible to find Dexia adults in the field continually during the period between the first and last findings of each generation. The September 12 record of 1928 was the capture of a single adult. There are three recorded generations in Chosen, and the presence of this fly may possibly be an indication of the occurrence of a third brood in this country. However, since it was the only specimen taken in three seasons, scouting during this period of the year, the occurrence of a third generation here seems doubtful.

CAGE EXPERIMENTS

MATING

Mating has never been observed in the field, and only once under cage conditions. During 1927 and 1928 over a hundred mating experiments were tried, different types and sizes of cages being used under different conditions of light, temperature, and humidity, including some work with different velocities of air currents. The only success obtained was with a large field cage 8 feet square by 6 feet high, having a top of white muslin and sides of black mosquito-netting. (Fig. 2.) The top and sides of the cage are separate sections so that it may easily be transported to the field and bolted together in a favorable location, such as a shady spot at the edge of a wood. The food supplied was a 10 per cent honey solution sprayed on the foliage within the cage.

In such a cage activity seems quite normal, and the flies do not exhaust their energy by trying to escape. The adults are apparently able to see black side walls better than white ones as they will fly to black and alight, whereas they try to fly through the white nettings, and their wings soon become frayed.

Several experiments with a cage population of 10 flies yielded no mating. With cage populations averaging 70 to 100 individuals and a sex ratio of 2 or 3 males to 1 female, the average fertility was

60 per cent. The largest lot available was slightly over 400 flies, of which 175 were females. Eighty-eight per cent of the females recovered from the cage were fertile. There is always a loss from spiders which succeed in gaining entrance no matter how carefully the cage is constructed.

Flies are retained in the mating cage from four to six days, and at the end of that time mating is assumed to be complete. In order to determine when most of the mating occurred, three or four females were taken from the cage each day and dissected. Prior to fertilization the immature eggs are contained in the ovarian tubules, not having descended into the ovarian sac, and the liquid within the brown

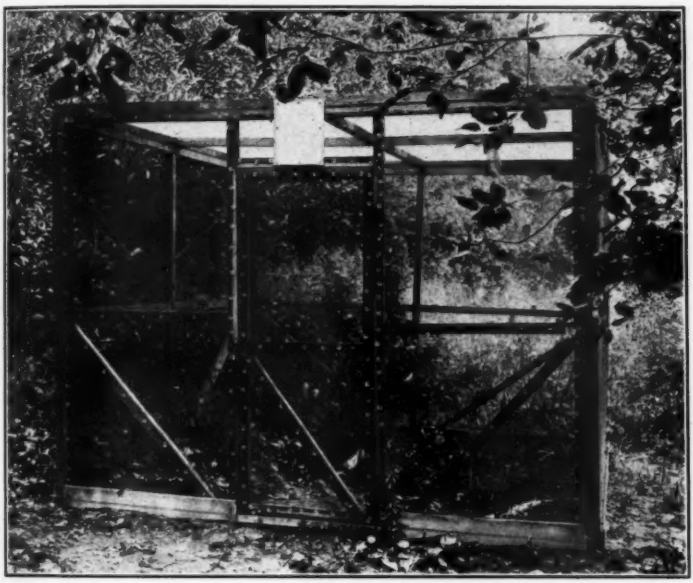

FIGURE 2.—Large portable field cage used for mating experiments

spermathecae is perfectly clear. After fertilization the contents of the spermathecae are white and turgid, and a number of eggs have descended into the ovarian sac. It was thus determined that the majority of the mating is accomplished by the end of the fourth day in the cage. In another series of experiments a number of flies were placed in the cage, and groups of them were removed at the end of 3, 4, and 6 days and placed in propagation jars. In this series the percentage of fertility was determined by post-mortem dissection. A small amount of mating does occur after the fourth day, but the increase in number of fertile females is not large enough to counterbalance the continual loss of flies resulting from the attack of spiders that enter the cage.

The optimum time of day for mating is unknown. In much of the cage work the flies were watched during their periods of activity but only one pair was observed in copula. This mating occurred during the twilight period. In one experiment a cage of flies was under continuous observation during all hours of visibility for three successive days, and no mating was observed, though later records showed that some of the flies had been fertilized.

PROPAGATION

Experimental propagation was attempted with this dexiid during 1927 and 1928. *Dexia ventralis* is normally a viviparous insect and when confined in a small jar will larviposit on soil without any apparent regard for the location of the host. The small planidium-like first-stage larvae work their way through the soil to the host and penetrate it. Many larvae may penetrate the same host (fig. 3), though usually only one develops. During warm weather host

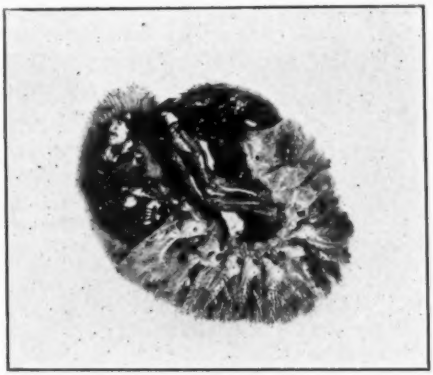

FIGURE 3.—Parasitized Japanese beetle larva showing heavy superparasitism. Each small black dot reveals the entrance point of a Dexia larva

grubs are very near the surface of the soil, and since the parasite larvae have very little soil distance to traverse, superparasitism is frequent.

To minimize superparasitism, a cage was developed consisting of a lantern globe with a flange on the bottom, which is closed with a 12-mesh copper-wire screen to retain the soil and yet allow the small larvae to pass through. The top is of white muslin fastened by a rubber band. (Fig. 4.) The cage contains 2 inches of moist loam and some blossoms of wild carrot (*Daucus carota*) held upright in a bottle of water. Fertile females are introduced and are fed daily by putting a few drops of honey solution on the flowers. The cages are placed on trays of soil containing host grubs. The larvae have to travel through at least 2 inches of soil before reaching their host, thus causing much greater scattering, and minimizing superparasitism. A renewal of host grubs is made by transferring the lantern globe to another previously prepared bottom tray. This method reduces individual handling of the adult flies to a minimum.

After a cage has been changed the bottom tray is not examined until the next day. This gives the larvae a chance to penetrate their host and pass through the first larval instar. Immediately after this

FIGURE 4.—The type of cage used for propagation work to secure parasitized Japanese beetle larvae

first molt the formation of the black spiracular sheath makes them readily observable. (Fig. 5). Before this molt they are discernible

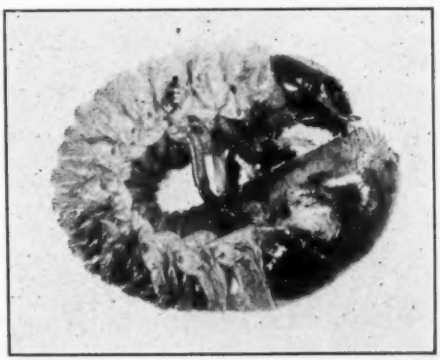

FIGURE 5.—Parasitized Japanese beetle larva showing the conspicuous black spiracular sheath of the Dexia maggot

only under the binocular microscope, but afterwards they can be seen with the unaided eye.

Parasitized hosts are sorted from the unparasitized and placed in screen-bottom cross-section trays with soil for rearing. Wheat is grown in these trays to furnish a source of food for the host.

Propagation of this species is still in the experimental stage. From one generation, before the technic outlined above was used, a number equivalent to the original stock was recovered, but no increase was obtained. In the second generation from this stock a ratio of increase of females of 6 to 1 was obtained by the methods just outlined. The third generation, in which the same stock was used, yielded 1 fly. The flies used were at least 50 per cent fertile, as determined by post-mortem dissection, but only 144 parasitized grubs were secured, and only 1 fly emerged. The cause of this "running out" of the stock was not determined. This same result has been noted with some species of Tachinidae.

LIFE HISTORY

The spring brood normally appears about the middle of June, and the first adults noted in the field are invariably males. The females are not seen until several days later. This observation is substantiated by records of emergence from shipments in which the peak of emergence of males precedes that of females by three or four days. Mating takes place presumably shortly after emergence, as the earliest females taken in the field are nearly always fertile. The period during which the larvae are retained in the uterus is short, and females begin larvipositing within 10 days after emergence. This act has never been observed in the field, and the time of maximum larviposition is unknown. One female captured in the field and held in the insectary began larvipositing a week later and continued doing so over a period of more than three weeks, until death. The larvae go at once into the soil in search of their host. The time necessary to effect entrance into the host is short. A few minutes usually sufficed in all instances noted.

The parasite larva has three instars, the first of which lasts only a few hours. After the first molt the spiracular funnel is formed, brown at first but soon turning black. The second and third instars are similar externally but can be distinguished by the shape of the larval mandibular hooks. The feeding period of larvae of this generation is from about 12 to 16 days, at the end of which time practically all the host tissue has been consumed except the integument and head capsule. (Fig. 6.) The parasite larva then draws away from its spiracular funnel, tears an irregular exit hole in the posterior ventral segments of its host, moves a short distance away and forms a puparium. (Fig. 7.)

The time spent in the puparium is usually from about 15 to 20 days. The young mature fly emerges and burrows to the surface of the soil, where it expands and dries and is ready to take wing in a few hours. The total elapsed time for the spring generation is from 36 to 42 days, as observed from a number of individuals reared in the insectary; this checks well with the appearance of generations in the field, the insectary time being only a few days shorter.

The summer generation emerges the first week in August. The mating and larvipositing habits are the same as noted for the previous generation. The young parasitic larvae develop until they have

FIGURE 6.—Parasitized Japanese beetle larvae showing their appearance when the Dexia larva is nearly full grown

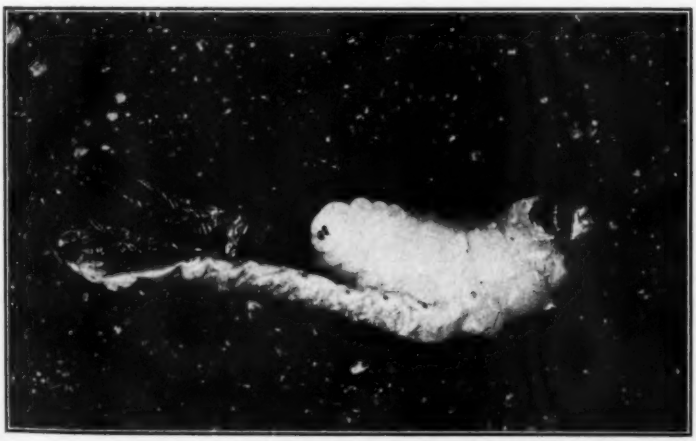

FIGURE 7.—Fully-grown Dexia larva emerging from its host

almost completed growth in the second stage. Their development then ceases, and the parasite overwinters within its host. The following spring the parasite resumes activity, changes to the third instar, completes its development within the host, and forms its puparium. The time spent within the puparium in the spring is longer than that of the other generation, but no exact figures are available.

The time for a complete cycle varies with the temperature. An insectary generation reared during late August, September, and October, 1927, averaged 45 days from the time of larviposition to adult emergence, which, with an added week for larval retention in the uterus, makes almost 8 weeks. Of the total parasitized grubs only about one-half yielded flies that fall, the rest overwintering.

The length of adult life is variable. Under insectary conditions the average life of females is three weeks. A single unfertile field-collected female lived 41 days in captivity. In the field adults are seldom found over any such period of time. Ten days to two weeks is the usual length of time during which the adults of any one brood may be seen in the field, and only once was an individual taken later than this (summer brood of 1927).

HABITS OF ADULTS

It has previously been noted that the adults of this species are crepuscular in habit. A number of adults confined in a large field cage were kept under continuous observation for two successive days. Observations were begun just before daylight and continued until after dark.

On both days the flies were motionless when first observed. The sun rose on these mornings about 4 a. m., eastern standard time. During the hour just before sunrise the flies showed some slight activity, which was chiefly walking. At sunrise activity increased, and the flies remained active until 6 a. m. The activity during this time consisted of walking, short darting flights, and feeding. About 6 a. m. activity began to decrease, and by 8 a. m. it had entirely ceased. The flies remained almost motionless the rest of the day except for an occasional cleaning movement of the legs. The entire day was bright and sunny. At about 5 p. m. the flies began again to exhibit slight activity and from 6.30 on activity increased rapidly until it reached a maximum at about 7 p. m. and continued so for three-quarters of an hour. Activity then decreased rapidly until dark, at 8.15 p. m., when the flies were again resting quietly as when first observed in the morning.

The flies were far more active during the evening period than during the morning, but this was probably due to the higher temperature at dusk. At sunrise the actinometer time was 3½ minutes. During the morning activity period, from sunrise to 6 a. m., the actinometer reading went from 3½ minutes to 20 seconds, and the temperature rose from 58° to 62° F. During the middle of the day the temperature rose to 83° and the actinometer time was 15 seconds. During the period of evening activity, 6.30 to 7.45 p. m., the actinometer went from 1 minute to no possible reading, and the temperature dropped from 82° to 71°. The results of the observations of the second day were practically identical. Weather conditions were similar, actinometer readings were the same, and the only difference was the temperature, which was about 3 degrees higher at any given time.

Observations made under natural conditions agree well with cage observations, though adults in the field remain hidden in the dense foliage during the quiescent periods. On dull, cloudy days adults can be found in the field nearly all morning, but they always disappear by 11 a. m. They have also been observed to be active in the field during periods of gentle rain, but as the rain increased they went to the underside of the leaves. Although adults may rest on almost any low-growing foliage in the field, they are found most frequently resting on the leaves of blackberry (*Rubus allegheniensis*). They have never been observed feeding in the field, though flowering plants and aphid colonies have been watched. It is probable that they are honeydew or nectar-gland feeders because their mouth parts are short and folding with broad flat labellae, not at all adapted for reaching into deep-flower nectaries.

BIOLOGICAL OBSERVATIONS

It has been noted that under natural conditions males precede females in emergence. Records on several shipments and all generations reared in propagation experiments show that the peak emergence of males precedes that of females by approximately four days.

The sex ratios from rearings is very nearly equal. It has never varied more than plus or minus 5 per cent in all cases recorded.

The length of effective life of first-stage Dexia larvae in the soil has not been definitely determined, but in one experiment some larvae, retained for six days in moist loam in a glass jar, were able successfully to penetrate hosts placed in the jar, though the percentage of larvae surviving until that time is not known.

Parasite larvae will penetrate and develop successfully in Popillia, but in a very limited series of experiments none of them could be induced to enter grubs of native Scarabaeidae, such as *Pachystethus lucicola* (Fab.), *Serica* sp., *Macrodactylus subspinosus* Fab., *Phyllophaga* spp., and *Strigoderma* sp. As only a few grubs of these other species were available these experiments are inconclusive.

The Japanese beetle may be parasitized in any of its larval stages, though parasitism is infrequent in other than the third instar. In propagation jars three larvae entered first-stage hosts. The effect on the hosts was greatly to prolong the first two stages, but both hosts and parasites survived all molts and the hosts eventually hibernated in the third stage. After overwintering one host was dissected, and the parasite larvae appeared normal. Another parasitized host died, and the third yielded an adult parasite. In all cases the spiracular funnel was retained.

Numerous hosts in the second stage have been parasitized in propagation jars. The effect of the parasitism has been to delay the molt. The parasite's spiracular attachment in *Dexia ventralis* is to the host derm, and the brown or black spiracular funnel is subcutaneous and very noticeable. After ecdysis more than half the host grubs lose any visible indication of parasitism. At first it was thought that the host had been able to free itself from the parasite, but it was soon found that the only thing lost was the spiracular sheath which remained attached to the old larval skin and was pulled out. There is no tear in the host tissue but the sheath is pulled out neatly, leaving a small, round, clean opening that readily escapes notice. On careful

observation the two anal spiracles of the parasite may be seen within the hole, coming into sight and retracting periodically. The parasite larva does not appear to be affected in any way by this loss of the sheath, and it completes its development as usual without another being formed.

The third stage of the host is the one most frequently parasitized, and the one which Dexia is considered to parasitize normally. In several cases in which propagation jars had hosts of all three stages, the third stage was parasitized almost to the exclusion of the others, though the possibilities offered by the different sizes of the host may influence the degree of parasitism. The time necessary for parasite development in second and third stage hosts is approximately equal.

Dexia larvae will also penetrate and develop in early prepupae if these are available. When this occurs the development of the host is arrested, and the host pupa is not formed. At this stage the host is undergoing histolytic action preparatory to pupation. Larvae developing in prepupae take one or two days less time when run in parallel series with others, and yield distinctly larger adults. Several cases were observed in which two individuals of Dexia matured in one host, a circumstance distinctly out of the ordinary for the species and never recorded from any stage except the prepupal.

No particular point on the host seems especially preferred for larval entrance, but the majority of entrances are dorsal. Some most unusual entrance points were noted in propagation work. One larva entered a host spiracle, and another penetrated the posterior thoracic proleg of the host at the point where the femur and tibia join. At the time this latter one was noted it was from 3 to 5 days old. It was apparently normal, and a sheath had been formed around it.

During the first two instars the parasite larvae lie at right angles to the longitudinal axis of the host within the body cavity, and feed on the body fluids and fatty tissue. The organs of the host are not consumed until later.

ADAPTABILITY TO AMERICAN CONDITIONS

Since Dexia has a variety of hosts in Chosen, each maturing at a different time of the year, the question arose as to whether in the United States suitable host material of Popillia would be present in the field during the periods when parasite larvae were being deposited. For the past three years diggings a foot or two square have been made weekly during the time that adults of Dexia were in the field. Usually five locations were dug, all near the colonization center and so chosen as to include high and dry situations which favor early emergence of the host and low, moist areas which tend to retard emergence. Suitable host stages may be considered from experimental evidence to be the second, third, and prepupal.

A summary of the three years' observations is presented in Tables 2, 3, and 4, in which are shown the number of Popillia grubs found of the various susceptible stages that would provide suitable host material for the different broods in the field in the periods specified. It will be seen that suitable host material is available in the field at all times, though in somewhat limited numbers during certain periods.

TABLE 2.—*Numbers of Popillia grubs found on the dates indicated that would serve as suitable hosts for the brood of Dexia ventralis that was in the field at Moorestown, N. J., from August 3 to 26, 1927*

Stage	Number of suitable host grubs found—				
	Aug. 4	Aug. 11	Aug. 19	Aug. 26	Sept. 2
Prepupae	9	3			
Last year's third stage	21	8	8	5	
Current third stage				8	83
Second stage	9	182	78	303	177

TABLE 3.—*Numbers of Popillia grubs found on the dates indicated that would serve as suitable hosts for Dexia ventralis, Moorestown, N. J., 1928*

Stage	Number of suitable host grubs found—							
	For brood 1, in the field from June 16 to 29, on—				For brood 2, in the field from Aug. 6 to 16, on—			
	June 20	June 27	July 5	July 11	Aug. 8	Aug. 15	Aug. 22	Aug. 29
Prepupae	6	2	2	1				
Last year's third stage	30	11	10	5	1			
Current third stage							4	24
Second stage					9	73	106	22

TABLE 4.—*Numbers of Popillia grubs found on the dates indicated that would serve as suitable hosts for Dexia ventralis, Moorestown, N. J., 1929*

Stage	Number of suitable host grubs found—					
	For brood 1, in the field from June 11 to 26, on—			For brood 2, in the field from July 30 to Aug. 11, on—		
	June 13	June 20	June 27	Aug. 1	Aug. 8	Aug. 16
Prepupae	7	2				
Last year's third stage	6	14	8			
Current third stage						3
Second stage				10	61	40

In Chosen there is a different host for each generation of the parasite, and the theory has been advanced that the various hosts are necessary because the parasite larvae can reach maturity only in a host undergoing histolysis preparatory to pupation. Such a condition would tend to limit the usefulness of a parasite if introduced into a country where only one host was available.

Experimental evidence has accumulated which tends to prove that, under conditions present here, host histolysis is not a necessary prerequisite to parasite maturity. Parasites have been reared in the late summer and early fall from second and third stage hosts which would have given rise to beetles the following summer. They were reared under insectary conditions, and the hosts would not have begun to pupate for another two months even if kept under optimum conditions in a greenhouse. Stronger evidence is offered by two cases of

parasite larvae entering second-stage hosts, coming to maturity, and emerging without these hosts changing to the third stage. Host histolysis does, however, shorten the time necessary for larval development and apparently offers better feeding conditions, as shown by the results of rearing parasites in prepupae. The resulting flies were larger, and in several instances two flies matured in one host.

SUMMARY

Dexia ventralis Aldr. is abundant in Chosen (Korea), and is a larval parasite of a number of Scarabaeidae in that country.

The parasites are shipped to this country as larvae in host grubs, about 30 per cent being received alive. Emergence from the host and pupation usually take place while the shipments are en route. About 85 per cent of the parasites received in this condition in 1927 produced adults. Few flies were yielded by those still in the hosts on arrival.

Four colonies of *Dexia ventralis* have been liberated in this country, and one is apparently established. Recoveries of adults have been made consistently from this colony.

It is fairly easy to induce mating in captivity by using large cages. Propagation experiments have been unsatisfactory.

The parasite larva has three instars. Two broods are produced in this country, and the winter is passed as an immature maggot within the host.

The adults are active mainly during the twilight periods and on cloudy days.

Larvae of *Dexia ventralis* can penetrate into and develop in larvae of the host in all stages, including the prepupal, but third-stage hosts are preferred. Suitable host material is available at all times. Host histolysis is not necessary to the development of the parasite larvae, and the species can apparently maintain its existence on a single host.

STORAGE ROTS OF THE JERUSALEM ARTICHOKE [1]

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INTRODUCTION

Little careful work seems to have been done on the storage of tubers of the Jerusalem artichoke (*Helianthus tuberosus* L.). In 1880, Decaisne (*4*) [3] showed that the tubers of this plant lack the corky layer which covers the potato. Because of the thin periderm, the tubers lose moisture rapidly when they are exposed to the air at room temperature. European workers, who have done most of the scientific work on the Jerusalem artichoke, agree in their published reports that the tubers are difficult to keep by any common storage method, because of shriveling and susceptibility to storage rots. In France the crop is planted only where the winter is mild enough for the tubers to be harvested as needed.

Few American workers have published statements in regard to the storage of this crop. Sibley (*10*) and Cormany (*2*) report that the tubers kept satisfactorily when left in the ground through the winter. Sibley windrowed the tubers and then threw earth over them with a turning plow, while Cormany left the tubers in the ground where they were grown. The latter states that these tubers were firmer and less rotted than tubers stored in the root cellar. Shoemaker (*9*), however, reports that he stored tubers in burlap bags in a cold cellar in Washington from November, 1925, to March, 1926, and found no shriveling or rotting. The temperature in the cellar was always low but never got quite down to freezing. This writer suggests that there may be temperatures at which the fungi that cause the rotting of the tubers in storage are quiescent.

The investigation reported in this paper was started in the fall of 1927, to secure more accurate information concerning the rots responsible for the heavy losses that occur when the tubers are stored. The objects of the investigation were three: (1) To determine the amount of rot which would develop when the tubers were stored under various conditions of temperature and humidity; (2) to find what organisms are responsible for these rots; and (3) to ascertain the effect of temperature on the development of the rots produced by various organisms.

[1] Received for publication Feb. 26, 1931; issued September, 1931. Paper No. 1007 of the Journal Series of the Minnesota Agricultural Experiment Station. A parallel study of the carbohydrate transformations occurring in the tubers during storage was carried on by the Divisions of Horticulture and Biochemistry. Their report was published separately (*11*).

[2] The writer is indebted to Dr. H. P. Traub for suggesting the problem and for assistance in certain phases of the work, and to Dr. E. C. Stakman for suggestions during the progress of the work and for advice in the preparation of the manuscript.

[3] Reference is made by number (italic) to Literature Cited, p. 351.

MATERIAL AND METHODS

Tubers of the Portland White variety of Jerusalem artichoke obtained from the Division of Horticulture were used in the experiments. The tubers were dug in the fall of 1927 after frost had killed the tops of the plants. Soon after the tubers were dug they were placed in strong duck vegetable bags and stored under different conditions of temperature and humidity. Data were taken on the various lots of tubers at intervals during the winter months, and all rotted or moldy tubers were removed when the examination was made. One of the experiments with these tubers was ended on March 29, 1928; a second was continued until June 7, 1928.

Whenever rotted tubers were removed from storage in the course of the experiments, an attempt was made to isolate the organisms that caused the rots. In making the isolations, the entire rotted tuber was first surface sterilized from three to five minutes in 1-1,000 mercuric chloride solution. It was then washed in three changes of distilled water, and a large block of the inner tissue was removed and surface sterilized for one-half minute in 1-1,000 mercuric chloride. After being washed in three changes of distilled water, the outer surfaces on all sides of the block were cut off with a flamed scalpel. The small freshly exposed block of tissue was then transferred to a sterile Petri dish and cut into fragments aseptically. The tissue fragments were plated out on acidified potato-dextrose agar, four or five fragments as a rule being placed in each Petri dish.

After 24 or 48 hours, the organisms which developed from the fragments were transferred to agar slants in test-tubes. Transfers to agar slants were always made from the edges of the fungous colonies. After several days, the tube cultures were checked over and only those which appeared to contain pure cultures of the organisms were saved. Certain of these cultures, together with other fungi and bacteria which it was thought might be capable of rotting artichoke tubers, were used in the inoculation experiments.

Two types of inoculation experiments were performed. In the first, in which only the bacterial cultures were used, the organisms from agar slants were streaked on the surface of sterile slices of artichoke tubers in Petri dishes. In the preparation of the slices for inoculation, the whole tubers were immersed for 15 minutes in 1-20 Semesan Bel and then washed in distilled water before being cut into slices with a flamed scalpel. The slices were placed on moist filter paper in Petri dishes which had previously been autoclaved for 30 minutes at 15 pounds pressure.

In the second type of inoculation experiment, in which both bacterial and fungous cultures were used, the "well method" described by Harter, Weimer, and Adams (7) was employed. The tubers were washed in running tap water, surface sterilized for one minute in 1-1,000 bichloride of mercury, and then washed in three changes of distilled water. A hole approximately one-half inch in depth was made in each by means of a flamed cork-borer one-half inch in diameter. The hole was then filled with the mycelial growth of the proper fungus or a suspension of the proper bacterium. After the inoculation was completed a flamed cover slip was sealed over the opening by placing petrolatum around the edges. The tubers were then distributed at different temperatures. After 10

days in certain cases and 20 days in others, the tubers were brought into the laboratory, cut in half lengthwise, and the amount of rot was recorded.

EXPERIMENTAL RESULTS

STORAGE EXPERIMENTS

Tubers of the Portland White variety of Jerusalem artichoke were dug in the fall of 1927 and placed in strong duck vegetable bags. On November 3, the bags, containing from 150 to 175 tubers each, were distributed to the following places of storage: (1) Common above-ground storage house, (2) root cellar, and (3) cold-storage house. At weekly intervals during the storage period the temperatures of the various storage places were recorded and the relative humidity determined by means of a sling psychrometer. The ranges of temperature and relative humidity during the storage period are indicated in Table 1.

TABLE 1.—*Percentage of sound, shriveled, and rotted Jerusalem artichoke tubers occurring under different storage conditions, University Farm, St. Paul, Minn.*

[Lots stored Nov. 3, 1927, to Mar. 29, 1928, and examined Jan. 30 and Mar. 29, 1928]

Storage conditions			Tubers stored originally	Date of inspection	Condition of tubers expressed as percentage of original numbers			
Tubers kept in—	Temperature	Relative humidity			Sound	Shriveled	Slightly rotted	Badly rotted
	° *F.*	*Per cent*	*Number*	1928				
Common storage house.	35–50	74–86	173	Jan. 30	27.74	38.73	16.19	17.34
				Mar. 29	7.51	6.94	41.62	43.93
Root cellar	35–40	89–92	150	Jan. 30	48.67	33.33	13.33	4.67
				Mar. 29	47.33	33.34	14.66	4.67
Cold storage	32–35	89–92	170	Jan. 30	85.29	10.01	4.70	----------
				Mar. 29	80.59	14.12	5.29	----------

On January 30, 1928, the three lots of tubers were checked over and the number of sound, shriveled, and rotted tubers was determined. All tubers showing rot were removed and the bags were then returned to the respective places of storage. On March 29, 1928, the three lots of tubers were checked over again and the experiment ended.

Percentages were determined from the number of sound, shriveled, and rotted tubers found on each date. These are shown in Table 1. The data in Table 1 show that the tubers kept satisfactorily only in the cold-storage house, where the temperature ranged from 32° to 35° F. and the humidity from 89 to 92 per cent. Under these conditions 80.59 per cent of the tubers were sound and not shriveled on March 29 when the experiment was ended and only 5.29 per cent of the tubers showed slight rotting. None was badly rotted.

In the root cellar where the temperature ranged from 35° to 40° F. and the relative humidity from 89 to 92 per cent, the tubers kept only fairly well. On March 29, 47.33 per cent of the tubers were still sound, 33.34 per cent were shriveled, 14.66 per cent were slightly rotted, and 4.67 per cent were badly rotted. In the common above-ground storage house, where the temperature ranged from 35° to 50° and the relative humidity from 74 to 86 per cent, only 7.51 per cent

of sound tubers remained on March 29, while 85.55 per cent of the tubers were rotted. More than one-half of these were badly rotted. The appearance of the three lots of tubers when they were removed from storage on March 29 is shown in Figure 1.

At intervals during the period of storage, samples were removed from the lots of tubers and their moisture content was determined. Such determinations were made on November 3 when the tubers were placed in storage, on December 20, on January 30, and on March 22. The data secured are summarized in Table 2. It will be seen from the table that under cold-storage and root-cellar conditions the moisture loss was slight. There was a difference of only 3.56 and 3.12 per cent, respectively, on a green-weight basis between November 3, 1927, and March 22, 1928. Under common storage conditions, on the other hand, there was a consistent decline in moisture content during the entire storage period, which amounted finally to 10.77 per cent on a green-weight basis.

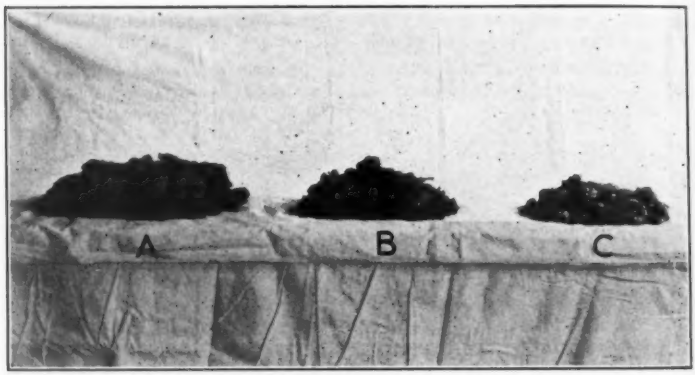

FIGURE 1.—Three lots of Jerusalem artichoke tubers as they appeared when removed from storage March 29, 1928, after having been stored November 3, 1927. Tubers from the lot in cold storage (A) were sound and in good condition; those from the lot stored in a root cellar (B) were slightly shriveled, but in fair condition; those from the lot kept in common storage (C) were badly rotted and shriveled, with Rhizopus, Penicillium, and Fusarium growing on their surfaces

TABLE 2.—*Variations in moisture content of Jerusalem artichoke tubers stored under different conditions from November 3, 1927, to March 22, 1928, at University Farm, St. Paul, Minn.*

Storage conditions			Date of determination	Percentage of green weight as—	
Tubers kept in—	Temperature	Relative humidity		Moisture	Dry matter
	° *F.*	*Per cent*			
Common storage house	35–50	74–86	Nov. 3, 1927	79.83	20.17
			Dec. 20, 1927	76.82	23.18
			Jan. 30, 1928	74.28	25.72
			Mar. 22, 1928	69.06	30.94
Root cellar	35–40	89–92	Nov. 3, 1927	79.83	20.17
			Dec. 20, 1927	78.07	21.93
			Jan. 30, 1928	77.99	22.01
			Mar. 22, 1928	76.71	23.29
Cold storage	32–35	89–92	Nov. 3, 1927	79.83	20.17
			Dec. 20, 1927	78.78	21.22
			Jan. 30, 1928	77.69	22.31
			Mar. 22, 1928	76.27	23.73

In a second storage experiment, also started on November 3, 1927, 500 tubers of the Portland White variety were placed in burlap bags and stored in the root cellar, while 326 tubers of the same variety were placed in duck bags and stored in the cold-storage house. These bags were not opened until June 7, 1928, when counts were made to determine the numbers of sound, shriveled, and rotted tubers. The percentages determined from these data are summarized in Table 3. In this experiment 58 per cent of the tubers stored in the root cellar were sound on June 7. This is a slightly higher percentage of sound tubers than was found in cold storage on the same date. However, the percentage of rotted tubers and the ratio of badly rotted to slightly rotted tubers was considerably higher in the root cellar.

TABLE 3.—*Percentage of sound, shriveled, and rotted Jerusalem artichoke tubers occurring under different storage conditions, University Farm, St. Paul, Minn.*

[Lots stored Nov. 3, 1927, to June 7, 1928, and examined June 7, 1928]

Storage conditions				Tubers stored originally	Condition of tubers expressed as percentage of original numbers			
Tubers kept in—	Temperature	Relative humidity	Container		Sound	Shriveled	Slightly rotted	Badly rotted
	° F.	*Per cent*		*Number*				
Root cellar	35-45	89-92	Burlap bag	500	58.00	17.60	6.80	17.60
Cold storage	32-40	89-92	Duck bag	326	53.10	28.80	10.74	7.36

Moisture content determinations were made for each lot of tubers at the beginning and end of the storage period. The percentages are presented in Table 4. The data in this table show that during more than seven months' storage under root-cellar conditions the sound tubers lost only 0.81 per cent of their green weight. The loss under cold-storage conditions was slightly higher, amounting to 2.61 per cent.

TABLE 4.—*Variations in moisture content of Jerusalem artichoke tubers stored under different conditions from November 3, 1927, to June 7, 1928, at University Farm, St. Paul, Minn.*

Storage conditions				Date of determination	Percentage of green weight as—	
Tubers kept in—	Temperature	Ralative humidity	Container		Moisture	Dry matter
	° F.	*Per cent*				
Root cellar	35-45	89-92	Burlap bag	Nov. 3, 1927	79.83	20.17
				June 7, 1928	79.02	20.98
Cold storage	32-40	89-92	Duck bag	Nov. 3, 1927	79.83	20.17
				June 7, 1928	77.22	22.78

ORGANISMS ISOLATED FROM THE ROTTED TUBERS

Tissue-fragment isolations were attempted from many of the rotted tubers which were removed from storage in the course of the experiments. Two species of Rhizopus were the organisms commonly found growing and fruiting on the tubers when they were removed

from storage on January 30 and on March 29. A species of Penicillium and a species of Fusarium were also present on the surface of some of the tubers but the two species of Rhizopus were the only fungi secured from the tissue-fragment cultures.

Preliminary inoculations were made and one species of Rhizopus was shown to be a vigorous parasite. This was later identified as *R. nigricans Ehr.* The other species, which was determined as *R. tritici* Saito, proved to be only weakly pathogenic at temperatures below 20° C. Figure 2, A, shows typical rotted tubers with *R. nigricans* growing on the surface. This figure also shows (B) the same tubers

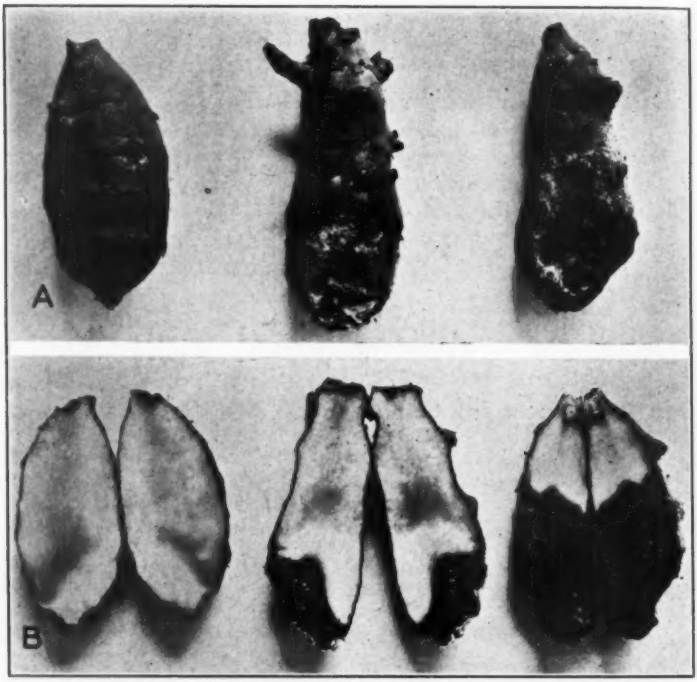

FIGURE 2.—Jerusalem artichoke tubers rotted by *Rhizopus nigricans;* surface views are shown at A, and B represents the same tubers cut lengthwise to display the soft dark brown rot caused by this fungus; the tuber at the left is sound

cut lengthwise to display the dark-brown soft rot associated with this organism. The same type of rot is shown, in Figure 6, to result from inoculation with pure cultures of *R. nigricans.*

A grayish bacterium developed from the fragments in a number of plates after cultures of Rhizopus had been secured. This organism appeared to be a secondary invader of the rotted tissue, and tests of its pathogenicity showed that it was unable to rot the tubers when it was introduced by needle streaks. However, when large amounts of inoculum were placed in wells in the tubers, a slight rotting occurred. Later, this organism was stained by Gray's method (*5*) and was found

to have polar flagella. It was then grown on various media and identified as *Pseudomonas fluorescens* (Flügge), migula.[4]

Pure cultures of the Penicillium species and of the Fusarium species mentioned earlier were obtained by direct transfers of spores and mycelium from the surface of rotted tubers. Cultures of the Penicillium were identified by Charles Thom, of the Bureau of Chemistry and Soils, United States Department of Agriculture, as belonging in that section of the Penicillia characterized by fasciculate conidiophores and closely related to *P. cyclopium* and *P. palitans* of Westling. Cultures of the Fusarium were identified by C. D. Sherbakoff, of the Tennessee Agricultural Experiment Station, as belonging in the section Roseum of the Fusaria and probably as being *Fusarium acuminatum* Ell. et Ev. emend. Wr. which occurs commonly on decaying roots and tubers. These organisms appeared to be only secondary invaders of rotted tissue, but subsequent tests proved that under favorable conditions they were weakly parasitic.

At the end of the second storage experiment on June 7, *Botrytis cinerea* Pers. was found most commonly on the surface of the rotted tubers. The Penicillium species was also present on the surface of some of the tubers, but tissue-fragment isolations from 16 of the rotted tubers all yielded the Botrytis.

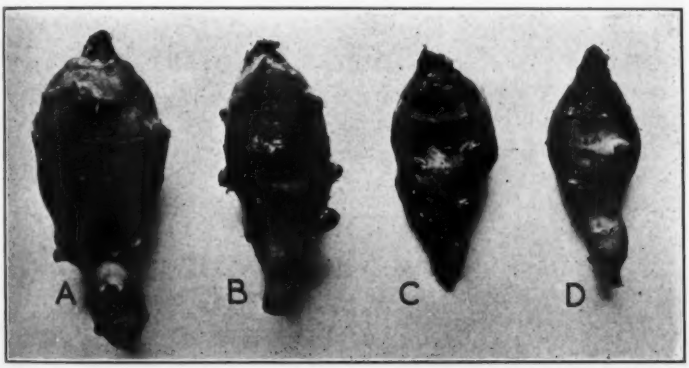

FIGURE 3.—Jerusalem artichoke tubers showing the light brown, slightly sunken lesions caused by *Botrytis cinerea*; infection has taken place through a raised lateral bud in A and B, and through a wound at the end of C and D

Some of the rotted tubers on the surface of which *Botrytis cinerea* was fruiting were washed, and definite, light-brown, slightly sunken lesions were found marking the extent of the rot. Such lesions are shown in Figure 3, infection having taken place through a raised lateral bud on the two tubers at the left and through a wound at the ends of the other two. After the tubers were washed they were placed in a moist chamber for 48 hours. At the end of this time the rotted areas were covered with a fluffy, white growth of mycelium, which soon changed to a powdery brown as the Botrytis began to form conidia abundantly. Figure 4 shows this mycelial growth (A) and also the same tubers cut lengthwise (B) to display the light-brown

[4] The writer is indebted to Delia Johnson for assistance in identifying this organism.

soft rot associated with *B. cinerea*. Inoculations made later with pure cultures of this fungus resulted in a light-brown soft rot exactly similar in appearance to that shown in Figure 4, B.

A summary of the results of all the isolations attempted shows that *Botrytis cinerea* and *Rhizopus nigricans* were the organisms secured from the largest number of rotted tubers. The other three fungi, while they occurred rather frequently on the surface of the rotted tubers, were isolated in only a relatively small number of cases.

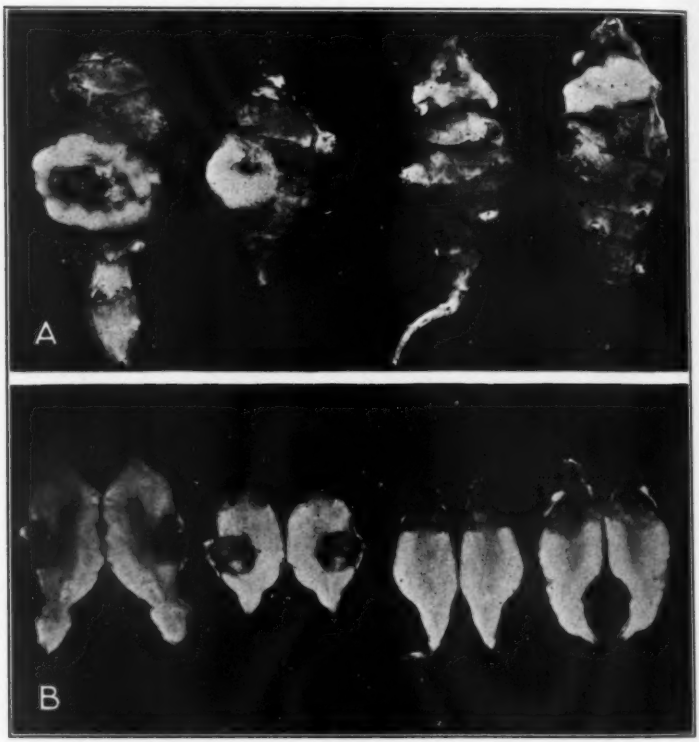

FIGURE 4.—Jerusalem artichoke tubers rotted by *Botrytis cinerea*; surface views are shown at A, and B represents the same tubers cut lengthwise to display the light-brown soft rot caused by this fungus

Apparently they were unable to compete with *B. cinerea* and *R. nigricans* in their growth through the tissue.

Pseudomonas fluorescens developed in a number of the plates but always from fragments which also yielded a fungous growth. Since soil is a common habitat of this bacterium, (*1*) it is probable that it was present on the surface of the tubers when they were dug and grew into them only after rot had begun. As will later be shown, this organism is unable to attack sound tubers when introduced into shallow wounds.

EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF TUBER ROTS

A preliminary test of the pathogenicity of some of the organisms isolated from the rotted tubers was made at 20° C., a temperature which was believed would be favorable for the development of the rots. Two isolations of *Rhizopus tritici*, two of *R. nigricans*, the Fusarium species, and *Pseudomonas fluorescens* were included in this test. The inoculations were made by the well method. After 10 days at 20° the tubers were cut lengthwise and notes were taken on the amount of rot. Both isolations of *R. nigricans* caused a severe rotting of the tubers at this temperature, whereas the two isolations of *R. tritici* caused only a slight rot. The Fusarium species also caused a slight rot, while *P. fluorescens* had not attacked the tubers after 10 days at this temperature.

Further experiments were then made to determine the effect of temperature on the rots produced by the organisms mentioned above and also by the Penicillium species, *Sclerotinia sclerotiorum* (Lib.) Massee, *Sclerotium rolfsii* Sacc., *Bacillus carotovorus* Jones, and *B. aroideae*[5] Town. *Botrytis cinerea* had not been isolated at the time these tests were made and hence was not included.

Two methods of inoculation were used. In the first, employed only with the bacterial cultures, streaks were made across the surface of sterile slices of artichoke tubers on moist filter paper in Petri dishes. The dishes were then distributed at various controlled temperatures. The checks for each temperature were treated like the inoculated slices except that the streaks were made with a sterile needle. The temperatures employed were approximately 2°, 6°, 13°, 20°, 27°, and 35° C. After 10 days at these temperatures the dishes were removed and the amount of rot recorded. The results are given in Table 5 and illustrated in Figure 5.

TABLE 5.—*Severity of rot produced in slices of Jerusalem artichoke tubers inoculated by the streak method with Bacillus carotovorus, B. aroideae, and Pseudomonas fluorescens and left at 2°, 6°, 13°, 20°, 27°, and 35° C. for 10 days*

Organism	Severity [a] of rot after 10 days at temperatures indicated in ° C.					
	2°	6°	13°	20°	27°	35°
Bacillus carotovorus	1	1	1	3	2	1
Bacillus aroideae	1	1	1	3	3	3
Pseudomonas fluorescens	0	0	0	0	0	0
Check	0	0	0	0	0	0

[a] 0=no rot; 1=slight rot; 2=moderate rot; 3=severe rot.

This experiment showed that *Bacillus carotovorus* and *B. aroideae*, two of the common soft-rotting bacteria, are able to rot Jerusalem artichoke tubers over a temperature range of 2° to 35° C. if introduced into shallow wounds. The optimum temperature for the development of the rots caused by these organisms was 20°, and severe rotting occurred only at and above that temperature. At temperatures above 20°, *B. carotovorus* produced much less rot than

[5] The cultures of *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* were obtained from J. J. Christensen; those of *Bacillus carotovorus* and *B. aroideae* were transfers from the cultures of J. G. Leach.

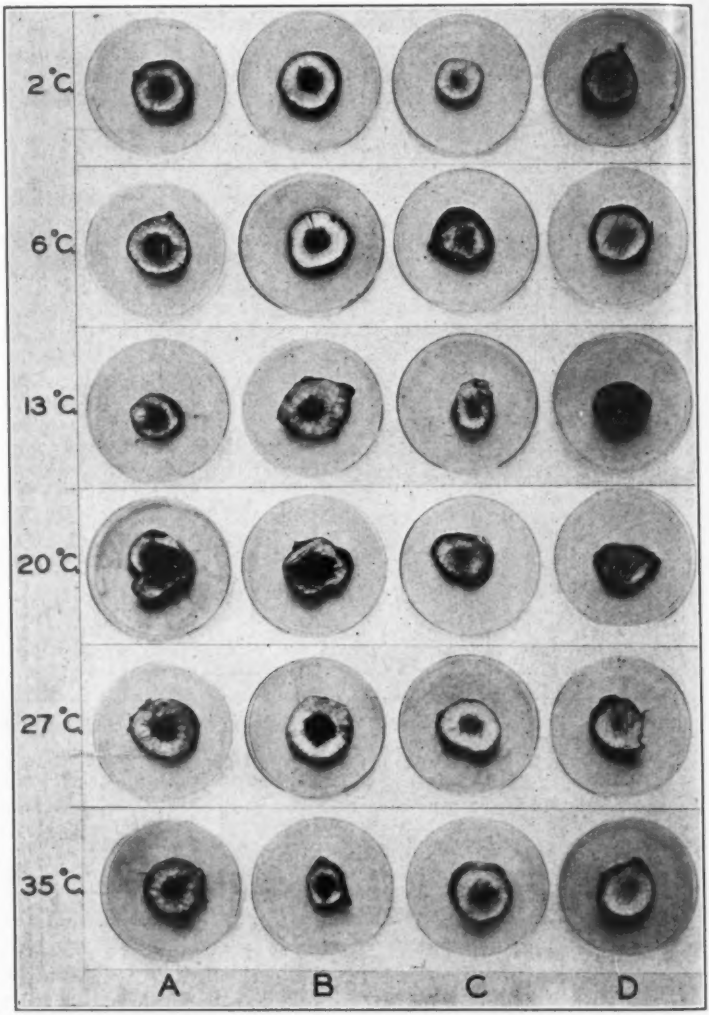

FIGURE 5.—Severity of rot produced in slices of Jerusalem artichoke tubers inoculated by the streak method with *Bacillus carotovorus* (A), *B. aroideae* (B), and *Pseudomonas fluorescens* (C) and left at 2°, 6°, 13°, 20°, 27°, and 35° C. for 10 days. The check slices (D) are free from rot at all the temperatures

did *B. aroideae*, which caused considerable rotting at both 27° and 35°. Under the conditions of this experiment *Pseudomonas fluorescens* isolated from rotted artichokes was unable to rot the slices at any of the temperatures tested. The checks remained sterile in all cases.

In the second set of inoculations the well method was employed. Tubers inoculated with cultures of the bacteria mentioned above were left at temperatures of approximately 2°, 6°, 13°, and 20° C. for 20 days. The check tubers which were placed at each temperature

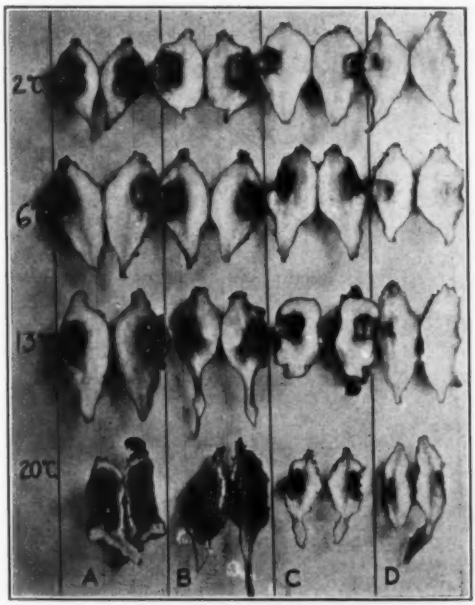

FIGURE 6.—Severity of rot produced in Jerusalem artichoke tubers when inoculated by the well method with *Bacillus carotovorus* (A), *B. aroideae* (B), and *Pseudomonas fluorescens* (C), and left at 2°, 6°, 13°, and 20° C. for 20 days. Check tubers (D) not rotted

were treated in the same way as the others, except that the well was filled with sterile artichoke decoction. After 20 days the severity of the rot which had developed was recorded. The results of a typical series of well inoculations with these organisms are presented in Table 6 and are illustrated in Figure 6. These results are in agreement with those secured in the streak inoculations, except that *Pseudomonas fluorescens* was able to cause a slight rotting of the tubers when large amounts of inoculum were introduced into the wells. Again there was no severe rotting of the tubers by any of the organisms below 20°.

TABLE 6.—*Severity of rot produced in Jerusalem artichoke tubers inoculated by the well method with cultures of various bacteria and fungi and left at 2°, 6°, 13°, and 20° C. for 20 days*

Organism	Severity [a] of rot after 20 days at temperatures indicated in ° C.			
	2°	6°	13°	20°
Bacillus carotovorus	1	1	1	3
Bacillus aroideae	1	1	1	3
Pseudomonas fluorescens	1−	1−	1−	1
Check 1	0	0	0	0
Rhizopus tritici Isolation 121	1−	1−	1−	2
Rhizopus tritici Isolation 140	1−	1−	1−	2
Rhizopus nigricans Isolation 125	1+	2	3	3
Rhizopus nigricans Isolation 133	1+	2	3	3
Check 2	0	0	0	0
Fusarium sp.	1−	1−	1−	1+
Penicillium sp.	1−	1−	1	2
Sclerotinia sclerotiorum	1	1	3	2
Sclerotium rolfsii	1−	1	1	3
Check 3	0	0	0	0

[a] 0=no rot; 1=slight rot; 2=moderate rot; 3=severe rot.

It appears from the results of the two inoculation experiments just discussed that bacterial rots of Jerusalem artichoke tubers might be expected at any storage temperature above freezing, but would be of importance only at temperatures considerably higher than those at which the tubers would ordinarily be stored.

The effect of temperature on the rots produced by the two species of Rhizopus, whose pathogenicity was established in the first set of inoculations at 20° C., was also tested. Tubers inoculated by the well method with isolations 125 and 133 of *R. nigricans* and with isolations 121 and 140 of *R. tritici*, were left at temperatures of approximately 2°, 6°, 13°, and 20° for 20 days. The severity of the rot which developed at each temperature during this time is shown in Table 6 and is illustrated in Figure 7. The more severe rotting produced by *R. nigricans* at all of the temperatures employed is quite evident. The maximum amount of rotting produced by this organism was at 13°. Rotting at 20° was practically as severe, although it appeared much less extensive because of the shriveling of the tubers at the higher temperature. *R. tritici* caused a moderate amount of rot at 20°., but only a very slight rot at the lower temperatures. It appears, therefore, that *R. nigricans*, which Harter and Weimer (*6*) have concluded is the most destructive member of the genus Rhizopus under natural conditions, may be responsible for much of the Rhizopus rot of Jerusalem artichoke tubers in storage.

A series of inoculations similar to these just described was employed to test the effect of temperature on the rots caused by *Sclerotinia sclerotiorum*, *Sclerotium rolfsii*, a species of Fusarium, and a species of Penicillium. The results obtained are presented in Table 6, and the rots at the various temperatures are illustrated in Figure 8. It is evident from the table and figure that all four of the organisms were able to rot the tubers over the entire range of temperatures tested. However, as in the inoculations with Rhizopus, severe rotting occurred only at 13° and 20° C.

The most extensive rot in this series of tests developed in the tubers which were inoculated with *Sclerotinia sclerotiorum* and held at 13° C.

This organism likewise caused a well-marked rot at the lower temperatures. A basal stem rot and tuber rot caused by this fungus is common on Jerusalem artichoke in New Zealand, according to Cunningham (*3*). It has been reported as a frequent cause of the rotting of artichoke tubers in storage, but was not isolated at any time during the course of the present experiments.

Higgins (*8*) has shown that *Sclerotium rolfsii* has its optimum temperature for growth between 30° and 35° C., and grows very poorly at temperatures below 20°. As was to be expected, there-

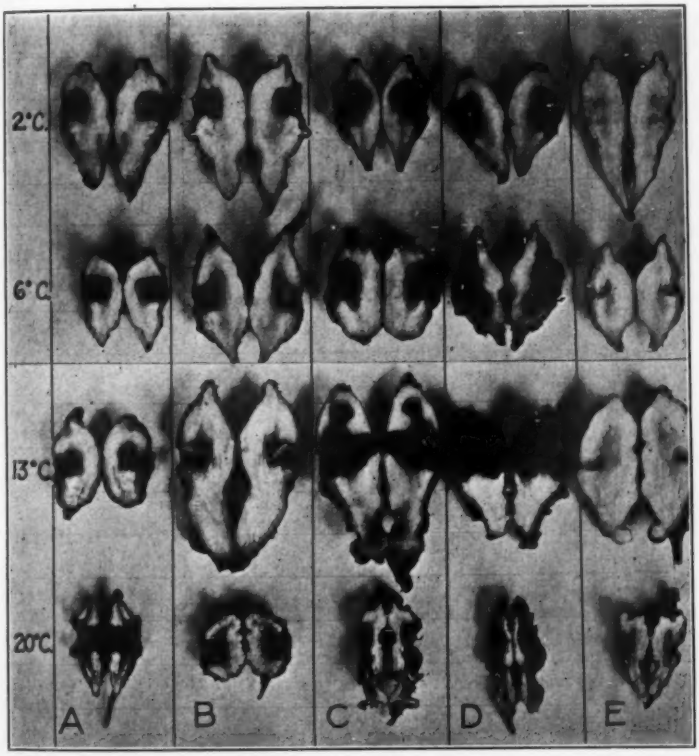

FIGURE 7.—Severity of rot produced in Jerusalem artichoke tubers when inoculated by the well method with isolations 121 (A) and 140 (B) of *Rhizopus trictici*, and with isolations 125 (C) and 133 (D) of *R. nigricans* and left at 2°, 6°, 13°, and 20° C. for 20 days. Check tubers (E) not rotted

fore, this organism produced little rotting of the tubers below 20°. At this temperature, however, a well-marked rot developed, as shown in Figure 8. It is evident that this organism would not be important as a cause of rot in artichokes stored at the temperatures usually employed.

The Fusarium species and Penicillium species both caused rot at all of the temperatures tested. The rot was slight in extent, however, except at 20° C. This temperature is, of course, considerably higher than any to which artichokes would be exposed in winter

storage under ordinary conditions. It appears probable, therefore, that these organisms are of importance as extenders of rots which have already begun, but not as primary exciters of rot when the tubers are stored at the usual temperatures.

SUMMARY

A study was made to determine the organisms responsible for the storage rots of Jerusalem artichoke tubers and to determine whether there were conditions of temperature and relative humidity unfavorable for the development of the rots under which the tubers could be stored successfully.

Storing the tubers at a temperature near the freezing point was shown to be the only successful method of keeping a large percentage of the tubers free from rot and shriveling for any length of time after they were dug. There was little rot and only slight shriveling when tubers were stored for five months at a temperature range of 32° to 35° F. and a relative humidity of 89 to 92 per cent.

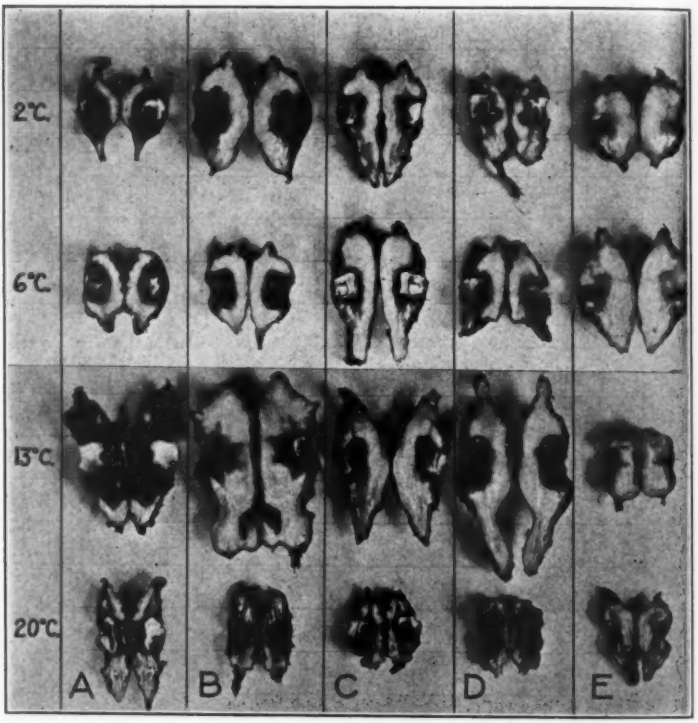

FIGURE 8.—Severity of rot produced in Jerusalem artichoke tubers when inoculated by the well method with *Sclerotinia sclerotiorum* (A), *Sclerotium rolfsii* (B), Fusarium species (C), and Penicillium species (D) and left at 2°, 6°, 13°, and 20°C. for 20 days. Check tubers (E) not rotted

High temperature and comparatively low relative humidity seem from these experiments to be the conditions most favorable for the development of the storage rots of Jerusalem artichoke tubers.

Botrytis cinerea and *Rhizopus nigricans* were the fungi isolated most frequently from the rotted tubers. *R. tritici*, a species of Penicillium, and a species of Fusarium, probably *acuminatum*, were isolated in a few cases. *Pseudomonas fluorescens*, a common soil-inhabiting bacterium, developed from fragments in many of the isolation plates.

Tests were made of the pathogenicity of all these organisms. *Rhizopus nigricans* proved to be a vigorous parasite and caused a rapid soft rot of the tubers at 6°, 13°, and 20° C. The other organisms caused only slight rotting at temperatures below 20°. The effect of *Botrytis cinerea* at temperatures below 20° was not tested because of the late date at which this fungus was first isolated.

Sclerotinia sclerotiorum, reported in the literature as causing a severe rotting of artichoke tubers in storage, was not isolated during these experiments. However, sound tubers were inoculated with this organism and it was found to cause a marked rotting of the tubers even at 2° C.

Inoculations were made with *Sclerotium rolfsii*, *Bacillus carotovorus*, and *B. aroideae* to determine whether these common, soft-rotting organisms might not also rot the tubers. All rotted the tubers over a temperature range of 2° to 20° C. However, only slight rotting occurred at temperatures below 20°.

Of the organisms whose pathogenicity was tested, *Rhizopus nigricans* and *Sclerotinia sclerotiorum* were the only two which caused a severe rotting of the tubers at temperatures within the range to which they would be exposed in cold storage or in the root cellar during the winter months.

Near the freezing point, the rots produced by these two organisms develop slowly, and successful storage of the tubers is possible.

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SUBERIZATION AND WOUND-PERIDERM FORMATION IN SWEETPOTATO AND GLADIOLUS AS AFFECTED BY TEMPERATURE AND RELATIVE HUMIDITY [1]

By ERNST ARTSCHWAGER, *Pathologist*, and RUTH COLVIN STARRETT, *Assistant Cytologist, Division of Sugar Plants,*[2] *Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

An essential condition for successful storage of sweetpotato roots and gladiolus corms is that injuries received during harvest be speedily repaired and that no exposed tissues remain to act as infection courts for pathogenic organisms.

In a study of the joint relation of temperature and relative humidity to infection of potato with various rot-producing fungi,[3] it was noted that infection apparently did not take place if the relative humidity was very high, but if the humidity was lowered infection developed rapidly. Artschwager [4] showed that wound cork in potato developed rapidly when the humidity was held practically at saturation, but that the development of this protective layer was much belated or even inhibited at the lower humidities. It occurred to the writers that a similar relation might exist between wound-cork formation and infection in the sweetpotato, especially since Weimer and Harter [5] had already published data showing a positive correlation between wound-cork formation and infection for the high humidities.

Lauritzen was unable to get infection of sweetpotatoes in the temperature range of 20° to 33° C. if the relative humidity was between 95 and 100 per cent, but if the relative humidity was dropped to 80 per cent, with temperature ranges as before, infection developed rapidly. According to Harter and Weimer,[6] wound-cork formation in sweetpotato is initiated after four days (actually, as will be shown later, wound cork is formed under optimum conditions after two days), within a temperature range of 19.5° to 33° with the relative humidity practically at saturation. The lack of infection at the higher humidities may be explained, therefore, by this rapid wound-cork formation which effectively shuts out pathogenic organisms. Since infection took place much more readily at the lower humidities, the question arose as to whether lower humidities were less favorable for wound-cork development, especially since positive observations to this effect had already been recorded for the potato and it was reasonable to suppose that other plants would react similarly to environmental changes.

The experiments reported in this paper were initiated to ascertain the effect of lower humidities on wound-cork formation in sweetpotato roots and gladiolus corms, but during the course of the investi-

[1] Received for publication Jan. 21, 1931; issued September, 1931.

[2] In cooperation with the Division of Horticultural Crops and Diseases, Bureau of Plant Industry.

[3] WEISS, F., LAURITZEN, J. I., and BRIERLEY, P. FACTORS IN THE INCEPTION AND DEVELOPMENT OF FUSARIUM ROT IN STORED POTATOES. U. S. Dept. Agr. Tech. Bul. 62, 36 p., illus. 1928.

[4] ARTSCHWAGER, E. WOUND PERIDERM FORMATION IN THE IRISH POTATO AS AFFECTED BY TEMPERATURE AND HUMIDITY. Jour. Agr. Research 35: 995-1000, illus. 1927.

[5] WEIMER, J. L., and HARTER, L. L. WOUND-CORK FORMATION IN THE SWEETPOTATO. Jour. Agr. Research 21: 637-647. 1921.

[6] WEIMER, J. L., and HARTER, L. L. Op. cit.

gation data were also gathered in regard to the effect of a much varying temperature and relative humidity range on both wound-cork formation and suberization.

MATERIALS AND METHODS

Controlled infection experiments with various storage-rot organisms [7] afforded an opportunity to obtain quantities of material for a study of suberization and wound-periderm development as affected by temperature and humidity. The sweetpotatoes were of the Yellow Jersey variety, grown on the Potomac Flats near Rosslyn, Va., across the river from Washington, D. C. Samples were first taken in March and again in October, 1928, some immediately after harvest and some after three weeks of curing; but the most extensive set of samples was obtained in February, 1929. For the most part the fleshy roots were halved at right angles to the growing axis, and blocks of tissue 5 by 10 by 40 mm., with the longest diameter traversing the vascular ring, were removed from the cut surface. The samples were dropped at once into an ordinary chrome acetic acid fixer, dehydrated, and embedded in paraffin. Sections were cut 15μ in thickness and stained with an ammoniacal gentian-violet stain as follows:

To 70 c. c. of a 1 per cent gentian-violet solution in 80 per cent alcohol add gradually 30 c. c. of aqua ammonia. Stain for several hours or leave the material in the stain overnight. Rinse in water and treat for a few seconds with an 8 per cent solution of hydrochloric acid; transfer to water and examine. If permanent preparations are desired, the specimens are dehydrated after staining and mounted in balsam.

The gladioli were mostly of the Maidens Blush variety, also grown on the Potomac Flats. The samples from which sections were cut for the determination of wound periderm were taken at 1-day intervals between January 6 and 27, 1929. Other samples were removed in March, 79 days after wounding.

OBSERVATIONS ON THE SWEETPOTATO

STRUCTURE OF THE SWEETPOTATO ROOT

The mature fleshy root of the Yellow Jersey variety of sweetpotato (pl. 1, A) shows in cross section a narrow cortex, protected by a periderm, and a central core of storage parenchyma in which numerous strands of vascular tissue are embedded. The continuity of the cambium ring which separates these two tissues is broken in places, permitting the passing out of root traces which traverse the fleshy root radially and connect with the lateral rootlets. What appear as additional breaks are in reality narrow invaginations of the cambium. The tissue inside of the cambium consists of a peripheral zone, in which the xylem elements show a typical radial arrangement, and individual bundles surrounded by a circular or partial cambium embedded in storage parenchyma. The cortex of the mature fleshy root is made up of secondary tissue. The cells composing it are large, starch-filled parenchyma cells interspersed with rows of sieve tubes with their companion cells and occasional latex vessels. The periderm is composed of thin-walled cells forming parallelepipeds with the

[7] The writers are under obligation to J. I. Lauritzen, of the Division of Horticultural Crops and Diseases, for conducting the storage-rot experiments for all the material used in these investigations.

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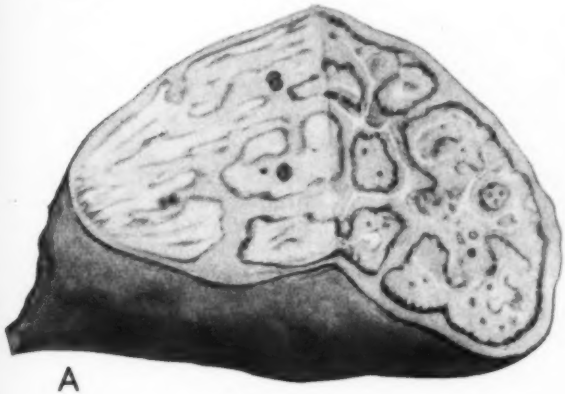

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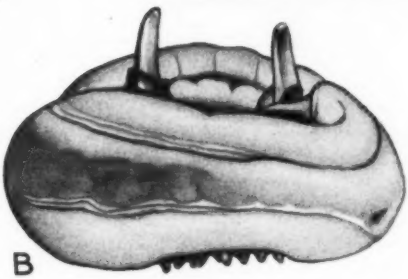

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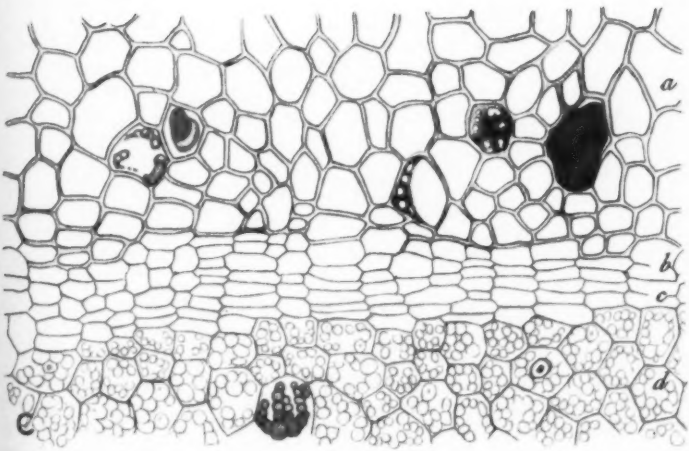

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A.—Fleshy root of sweetpotato cut to show transverse and tangential surfaces.

B.—Corm of Maidens Blush gladiolus.

C.—Suberization and wound-periderm formation in gladiolus. Suberized cells (*a*) and older periderm cells (*b*) stained blue. Newly formed periderm cells (*c*) and corm tissue (*d*) within periderm did not retain the ammoniacal gentian-violet stain. × 100

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shortest diameter in the radial axis of the organ. The outer periderm cells become partly lignified and are progressively sloughed off; but a phellogen layer remains active throughout the growing season, and through its activity the thickness of the protective periderm layer remains constant.

SUBERIZATION IN THE SWEETPOTATO

Priestley and Swingle [8] have shown that the cut surface of parenchymatous tissue is covered immediately with a fine layer of sap and broken cell fragments which offer a very suitable medium for the growth of microorganisms. Decay would follow rapidly if changes in the walls beneath the surface film did not develop a barrier to impede the progress of pathogenes.

A temporary, yet seemingly effective, blocking off of the exposed surface is secured through the suberization or "metacutinization" of the peripheral cell layers, a process which is greatly influenced by environmental conditions, especially temperature and humidity. In as short a period as 24 hours, under favorable conditions of high humidity and temperature, the walls of the outermost layer show with ammoniacal gentian-violet stain a purple color, while the adjacent tissue remains hyaline. Suberization progresses gradually and centripetally, but does not affect more than two or three cell layers (fig. 1, B), except in the region of the old periderm, where (fig. 1, A) a very broad suberized layer is formed. If conditions are unfavorable, i. e., at low humidity and temperature, desiccation of the surface cells soon takes place and a hard crust is formed, inhibiting suberization or greatly delaying it, so that no positive suberin test is obtained even after a period of a week or more.

The rate of suberization of cut fleshy roots of the Yellow Jersey variety of sweetpotato is shown in Figures 2, 3, and 4. At temperatures below 12° C. no detectable changes are produced at any of the recorded humidities. At temperatures slightly above 12° indications of suberization may be seen after six days (fig. 3), and at slightly lower humidities after 9½ days. (Fig. 2.) A further increase in temperature to 14.7° shows suberization after 5½ days (fig. 4), while an additional increase of only 1° reduces the time for suberization to be detectable to just 3½ days. (Fig. 3.) At temperatures above 20° evidence of suberization is seen after the second day, and with sufficiently high temperatures and humidity, even after the first day. Suberization at storage temperatures (between 28.7° and 34.8°), as seen in Figure 4, is more or less independent of relative humidities that are higher than 75 per cent, whereas lower humidities retard or even inhibit suberization in spite of favorable temperatures.

WOUND-PERIDERM FORMATION IN THE SWEETPOTATO

Cellular activity, as revealed by microscopic study, resulting in wound-periderm formation and coordinate with the gross anatomical changes, has been studied in many plants; and observations on the sweetpotato and the gladiolus show the procedure to be normal in most particulars. Soon after the root is cut or otherwise injured starch begins to disappear from the peripheral cell layers; the outer-

[8] PRIESTLEY, J. H., and SWINGLE, C. F. VEGETATIVE PROPAGATION FROM THE STANDPOINT OF PLANT ANATOMY. U. S. Dept. Agr. Tech. Bul. 151, 99 p., illus. 1929.

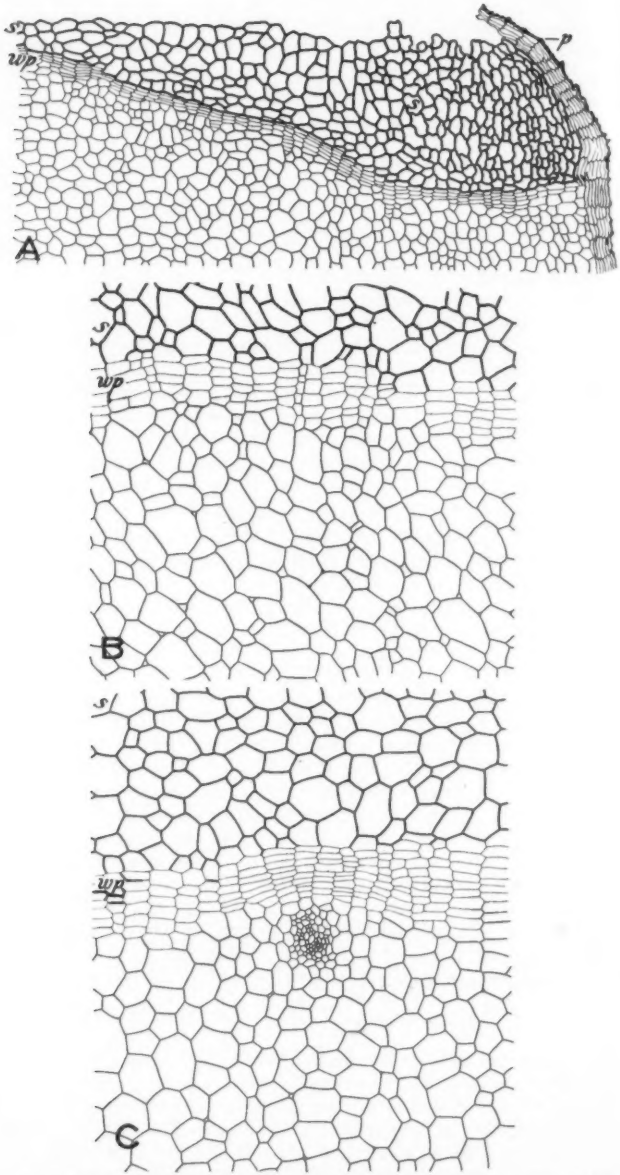

FIGURE 1.—Suberization and wound-periderm formation: A, In sweetpotato; greatest thickness of suberized region (*s*) near periderm (*p*), × 40; B, in sweetpotato, × 93; C, in gladiolus, × 93; *s*, suberized; *p* periderm; *wp*, wound periderm

most layer, however, composed mostly of cut or injured cells, retains its starch content. (Fig. 5, A.) Cell division now begins within the starch-free zone parallel to the cut surface, and gradually a continuous layer is formed across the cut surface below the suberin deposit, which functions as a cork cambium and which continues to divide, giving rise to a layer of wound periderm. (Fig. 5, A.) This is permanent and offers a more effective protection against the entrance of pathogenes than was afforded by the suberization of the peripheral cell layers. The contour of the cork cambium appears to be related to the types

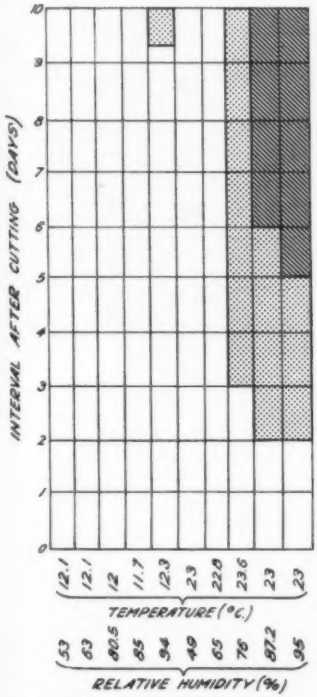

FIGURE 2.—Suberization (dotted) and wound-periderm formation (shaded) in sweetpotato, March, 1928

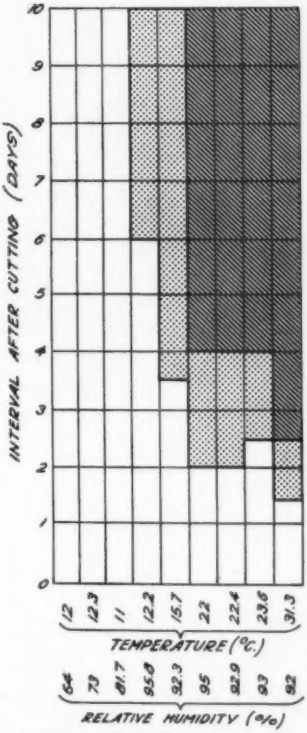

FIGURE 3.—Suberization (dotted) and wound-periderm formation (shaded) in sweetpotato, October, 1928

of tissues exposed in the cut. It is uniform in an uninterrupted zone of cortical tissue, but dips down more or less where it traverses elongated parenchyma cells between the vascular bundles, and rises close to the surface in the region of exposed vascular strands. Near the periphery of the cut root both suberin layer and wound periderm are sunk deeply into the tissue beneath the exposed surface. (Fig. 1, A.)

Since suberization precedes wound-periderm formation, the factors governing the former will also affect the second process. The lowest temperature at which wound-periderm formation was observed was 12.5° C. with a relative humidity of 93 per cent, but the process did

not start until 25 days after wounding. At 14.7° periderm formation was already evident after 10 days. (Fig. 4.) Increasing the temperature to 19.1° did not shorten the period necessary for this process, although suberization was observed at this temperature after 3½ days. However, a slightly greater increase in temperature reduced the period for wound-periderm development materially. At 21.7° wound periderm appeared after the fourth day, and at 31.7° after the second day. At the slightly higher temperature of 34.8° periderm develop-

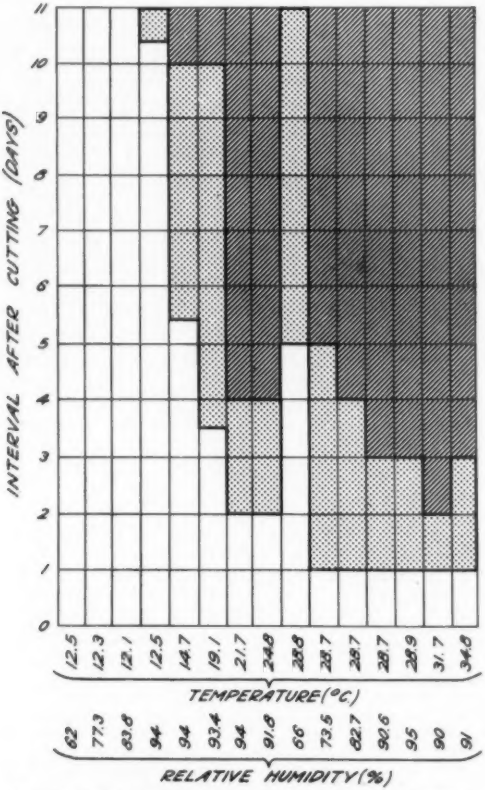

FIGURE 4.—Suberization (dotted) and wound-periderm formation (shaded) in sweetpotato, February, 1929

ment was retarded, while at still higher temperatures Weimer and Harter [9] state that the roots decayed before tests for periderm development could be made.

Variation in the relative humidity also influences periderm formation. At a relative humidity of 92.9 per cent and a temperature of 22.4° (fig. 3) wound periderm appeared after the fourth day, but at a relative humidity of 76 per cent no wound periderm had formed even

[9] WEIMER, J. L., and HARTER, L. L. Op. cit. (See footnote 5.)

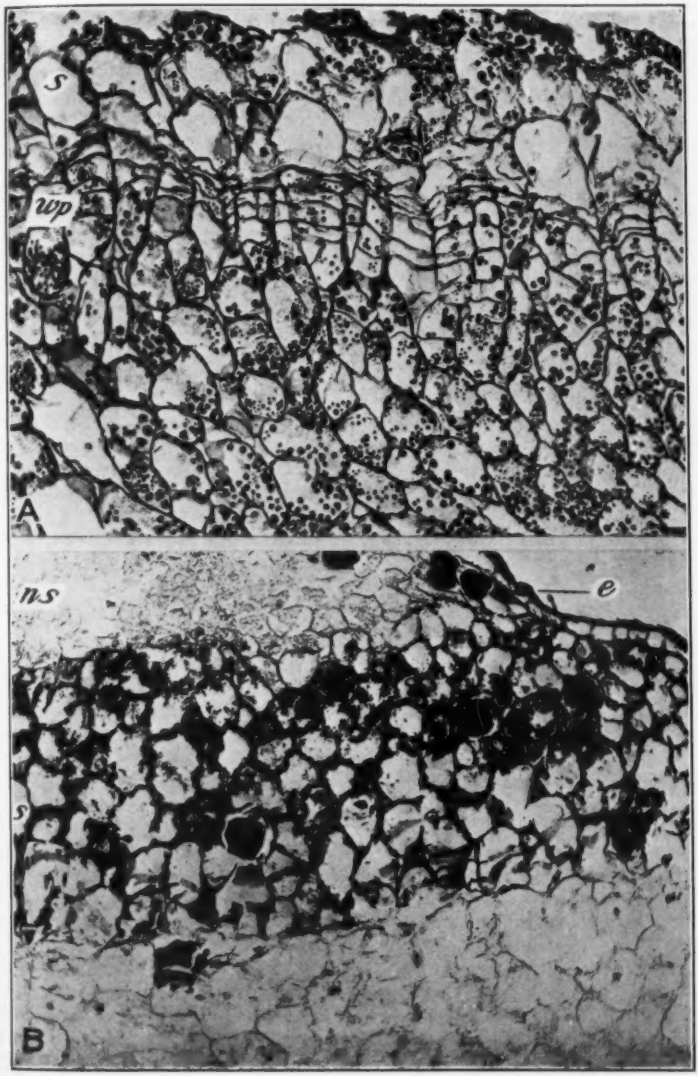

FIGURE 5.—A, suberization and wound-periderm formation in a fleshy root of sweetpotato kept for four days at a temperature of 28.7° C. and a relative humidity of 90.6 per cent; B, suberization in a corm of gladiolus kept for five days at a temperature of 28.3° and a relative humidity of 61 per cent; suberized zone broad and buried in tissue; *s*, suberized; *wp*, wound periderm; *ns*, nonsuberized. × 132

after 10 days at 23.6°. (Fig. 2.) The effect of variation in relative humidity on wound-periderm formation is best seen in Figure 4. At a relative humidity of 66 per cent no periderm had developed even after 11 days at 28.8°; at 73.5 per cent it appeared after the fifth day at 28.7°, and at 82.7 per cent after the fourth day at the same temperature; at relative humidities of 90 per cent and above, periderm appeared after the second and third days, at temperatures from 28.7° to 34.8°.

The experiments show that wound periderm will form readily at temperatures between 22° and 34.8° C., provided the relative humidity is sufficiently high. (Figs. 2, 3, and 4.) At lower temperatures the process is retarded and eventually inhibited, whereas, at temperatures above those used for curing, there is danger from decay. In control tests with cured fleshy roots practically identical results were obtained.

OBSERVATIONS ON GLADIOLUS

STRUCTURE OF THE GLADIOLUS CORM

In the gladiolus the mature corm, growing above the old one, is a depressed globose structure (pl. 1, B), protected by scaly leaves. Like other storage organs of its type, it is composed of parenchymatous tissue traversed by a number of vascular strands which in the central region of the corm are so close together as to form an almost axial core. The vascular bundles are at best only weakly developed. The xylem is limited to a few spiral vessels, while the phloem, somewhat more extensive, is composed of sieve tubes and relatively large companion cells. The corm tissue is protected by a single-layered epidermis covered with a well-developed cuticle, thus differing from the fleshy root of the sweetpotato, which has a protective layer composed of several rows of periderm cells.

SUBERIZATION IN GLADIOLUS

The first test for suberization in gladiolus was made 24 hours after wounding, but as, in certain corms, layers to the thickness of two cells below the cut surface retained the ammoniacal gentian-violet stain, it is probable that suberization had set in before the lapse of the first day. The suberization process in the gladiolus corm proceeds more vigorously than in the sweetpotato, and because of the uniformity of the storage parenchyma and the scarcity of vascular tissue the depth of the suberized layer is very regular. This singular uniformity is even evinced at the intersection of cut surface and epidermis, and the dip in the suberized layer which is so prominent in the sweetpotato (fig. 1, A) is wanting.

The rate of suberization and wound-periderm development for the different lots of the Maidens Blush variety of gladiolus is shown in Figure 6. Corms stored at temperatures ranging from 36.8° to 21.9° C. showed suberization after the first day, except one lot stored at a relative humidity of 61 per cent; and, as would be expected, the lots kept at the upper temperatures showed several layers suberized, while those at the lower temperatures exhibited the purple ammoniacal gentian-violet stain only in the outermost wall. At 19.3° suberization was evident after the second day, at 15.3° after the third day, at 12.5° after the fourth day, at 10° after the fifth day, and at 4.5° after the tenth day.

At sufficiently high relative humidities the suberization process in the gladiolus goes on normally, beginning in the outermost layer of the corm (pl. 1, C) and extending centripetally to a depth which is related to the storage temperature and the time of differentiation of the first periderm cells. At the lower relative humidities certain peculiarities were observed which were not met with in the sweetpotato. At the relative humidity of 75 per cent and a temperature of 12.4° C. the outermost layer of cells on the exposed surface remained unchanged.

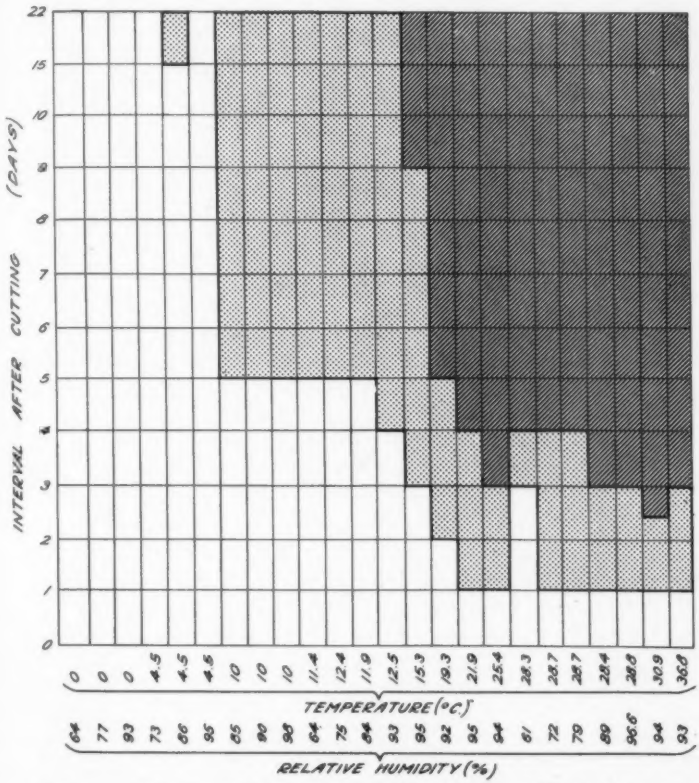

FIGURE 6.—Suberization (dotted) and wound-periderm formation (shaded) in gladiolus, January, 1929

Suberization was only evident below the second or third surface layer. At progressively lower relative humidities the failure of the surface cells to suberize became more marked, and in a lot stored at the relative humidity of 61 per cent and a temperature of 28.3°, as many as four or five layers of cells (fig. 5, B), did not change, so that the suberized zone was more or less buried in the tissue. A similar but even more striking case of "internal" suberization was observed in certain lots stored at relatively low temperatures for a period of 79 days. The relative humidities and the temperatures at which this

experiment was carried out, as well as the results obtained, are shown in Table 1. It will be seen that the nonsuberized layer was in many instances over 10 cells thick, as illustrated for the Chicago White

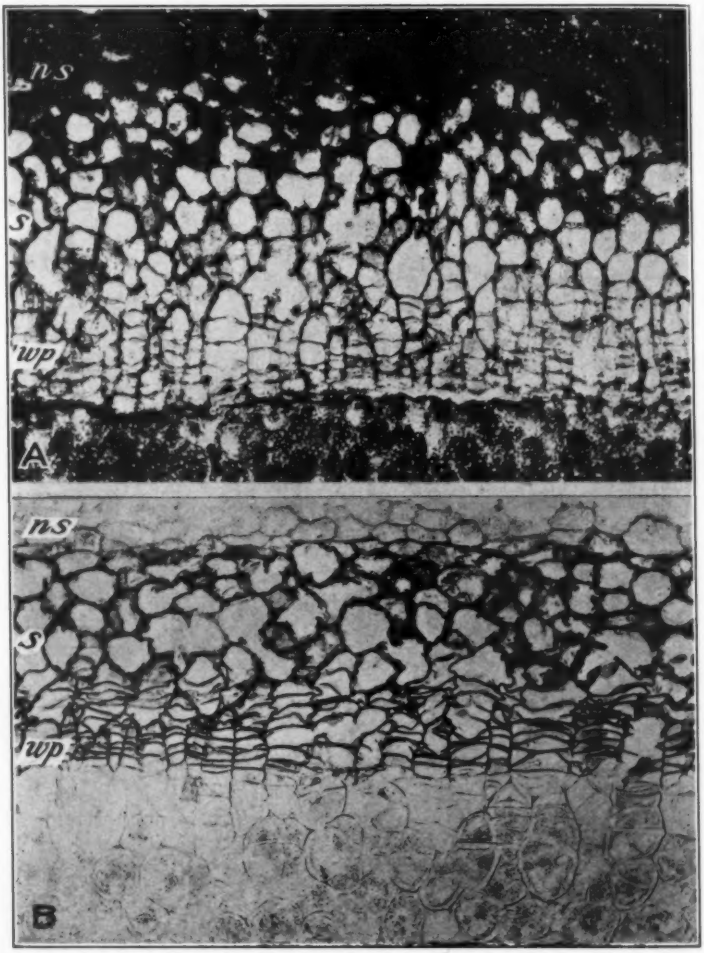

FIGURE 7.—Suberization and wound-periderm formation in gladiolus varieties: A, Chicago White, kept for 79 days at a temperature of 28.3° C. and a relative humidity of 95 per cent (both suberized layer and wound periderm buried in tissue); B, Latonia, kept for 79 days at a temperature of 10° and a relative humidity of 90 per cent (nonsuberized layer only two cells thick); *ns*, nonsuberized; *s*, suberized; *wp*, wound periderm. × 135

variety in Figure 7, A. The low temperature, although associated with a fairly humid atmosphere, probably will account for the great thickness of the nonsuberized layer, which must have dried out somewhat or become otherwise changed before suberization took place.

TABLE 1.—*Effect of temperature and humidity upon suberization and wound-periderm development in the gladiolus corm March, 1929, 79 days after wounding*

Temperature, ° C.	Relative humidity	Variety	Thickness of unchanged surface	Thickness of suberized layer	Wound cork
	Per cent		*Cell layers*	*Cell layers*	
10	85	Chicago White	3	6	Broad.
		Maidens Blush	6	6	Do.
		Virginia	5	5	Do.
		Latonia	1	5	Do.
10	90	Chicago White	1	4	Very broad.
		Maidens Blush	2	4	Do.
		Virginia	1	5	Do.
		Latonia	.5	4	Do.
10	98	Chicago White		2	Do.
		Maidens Blush	1	6	Do.
		Virginia	1	4	Do.
		Latonia	.5	4	Do.
4.5	73	Chicago White	20	5	Medium.
		Maidens Blush	20	8	Narrow.
		Virginia	21	4	Do.
		Latonia	15	4	Medium.
4.5	86	Chicago White	6	5	Do.
		Maidens Blush	16	8	Narrow.
		Virginia	6	7	Very narrow.
		Latonia	7	5	Do.
4.5	95	Chicago White	16	6	Narrow.
		Maidens Blush	5	6	Do.
		Virginia	2	5	Do.
		Latonia	2	6	Do.

WOUND-PERIDERM FORMATION IN GLADIOLUS

The development of wound periderm in gladiolus and the factors governing it were much the same as described for the fleshy root of the sweetpotato. It will be seen in Figure 6 that with 22 days as the time limit of the experiment the lowest temperature at which wound cork formed was 15.3° C. A gradual increase in temperature shortened this period until at 30.9° a cork cambium appeared after 2½ days. At the still higher temperature of 36.8° the differentiation of wound periderm was somewhat belated and proceeded at a less vigorous rate than at the slightly lower temperatures. Above 80 per cent, differences in relative humidity neither hastened nor slowed up periderm formation; but below 80 per cent, decrease in relative humidity is shown to have had a retarding influence. The effect of relatively low temperatures on wound-periderm formation is prominent. (Table 1.) In a previous section it has been shown that periderm did not develop at temperatures below 15.3° even after a period of 22 days, so that it might have been concluded that wound-periderm formation was completely inhibited at these temperatures. However, when observations were made after a period of 79 days, it was found that this supposition was only partly true and that a broad wound periderm would develop at 10° and at 4.5° if given sufficient time.

The wound cork of gladiolus and sweetpotato appears to belong to the common or "initial" type, although occasionally parenchyma cells are found in gladiolus containing a number of periclinal divisions suggestive of the "etagen" cork of the monocotyledons. Philipp [10] finds etagen cork in the majority of monocotyledons; but since little is said about wound cork in these plants and since normal and wound

[10] PHILIPP, M. UBER DIE VERKORKTEN ABSCHLUSSGEWEBE DER MONOKOTYLEDONEN. Bibliotheca Bot. Heft 92, 27 p., illus. 1924.

cork need not have the same origin, it would be premature to argue the significance of the occurrence of wound cork of the initial type in gladiolus.

SUMMARY

The fleshy roots of sweetpotato and the corms of gladiolus respond to wounding by a suberization process of the cut surface and a subsequent development of wound periderm. The course of both processes is greatly influenced by the surrounding medium, i. e., by the temperature and relative humidity of the air. If these conditions are favorable, the wound-healing process proceeds very rapidly; but under adverse conditions both processes may be inhibited or greatly delayed, as exemplified by a lot of gladiolus corms stored at a relatively very low temperature. These corms showed no suberization after 20 days, yet developed a normal wound periderm when left in storage for 79 days.

INHERITANCE OF TYPE OF FLORET SEPARATION AND OTHER CHARACTERS IN INTERSPECIFIC CROSSES IN OATS[1]

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INTRODUCTION

The type or manner of attachment of the oat spikelet to its peduncle and of the florets to their rachilla segments and the subsequent separation of the florets at maturity are highly important differentiating characters for the different species of oats. The mode of inheritance of these characters has been studied in crosses between *Avena fatua* and *A. sterilis* and between these species and several cultivated varieties of *A. sativa* and *A. byzantina*. *A. fatua* and *A. sterilis* are considered the prototypes of the majority of cultivated oat varieties. Similarities in structure and type of spikelet and in manner of floret separation in the cultivated and wild species are indications of relationship. Further evidences of relationship are indicated by the relative ease with which the wild and cultivated species intercross and by the apparently normal fertility of their hybrids.

REVIEW OF LITERATURE

SPIKELET AND FLORET SEPARATION

Surface (*9*),[3] in 1916, made an analysis of the mode of inheritance of several characters in crosses between *Avena fatua* and *A. sativa* var. Kherson. The "sucker mouth," or callus, at the base of the lower floret of the spikelet of *A. fatua*, resulting from separation by abscission, was found to be recessive to the pointed base of the lower floret of the spikelet of Kherson, resulting from separation by fracture. The rachilla-segment scar of the second or upper floret, resulting from separation by disarticulation, characteristic of Kherson, was completely dominant over that of the wild oat. A ratio of 1 Kherson type (cultivated) to 2 intermediate types to 1 *fatua* type (wild) was observed in F_2, indicating a 1-factor difference. The results in F_3 substantiated the findings in F_2.

Surface found several complete linkages in the wild oats, *Avena fatua*. The wild-type scar, or callus, occurred on the upper floret only when the lower floret was separated from the peduncle by abscission, leaving a similar but larger scar. The wild parent always had a strongly geniculate and twisted awn on both the lower and upper

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[1] The writer is indebted to T. R. Stanton, Senior Agronomist, and F. A. Coffman, Associate Agronomist, Division of Cereal Crops and Diseases, for valuable criticisms and suggestions, particularly with reference to terminology and description of varieties. Photographs and photomicrographs shown in this paper were produced by F. M. L. Foubert, Office of Information, U. S. Department of Agriculture.

[3] Reference is made by number (italic) to Literature Cited, p. 386.

florets. Pubescence on the rachilla segment of the second floret was shown to be completely linked with spikelet separation by abscission (wild base). The ring of hairs around the callus was shown to be completely linked with spikelet separation by abscission (wild base). Notwithstanding these findings, Surface states that "Evidence from certain other crosses, as yet not completely analyzed, indicates the existence of separate genes for some of these characters." He found a marked correlation between type of spikelet separation and pubescence on the lemmas in the F_2 plants. Partial linkage was noted between black color of glume and pubescence on the lemma of the lower floret.

Tschermak (*10*) has reported on crosses of *Avena fatua* × *A. sterilis* and on crosses of each of these wild forms with cultivated varieties. He found that the *A. sterilis* type of floret separation was completely dominant in F_1 hybrids of *A. fatua* × *A. sterilis*. A unit-factor difference was found in type of floret separation.

F_1 plants of *Avena sativa* × *A. fatua* and of *A. sterilis ludoviciana* × *A. sativa* were classed as intermediate. F_2 populations of both crosses segregated according to the ratio 9 intermediate to 3 *sativa* to 4 wild. Tschermak interprets his results by two alternative explanations—(1) by the assumption of a 2-factor (linked) difference between wild (*ABCABC*) and cultivated (*abCabC*) or (2) by the assumption of association—dissociation differences between wild ($\widehat{ABC}$ $\widehat{ABC}$) and cultivated ($A \downarrow B \downarrow C$ $A \downarrow B \downarrow C$).

Tschermak found complete linkage between articulate type of floret (as it occurs in *Avena fatua*) and fully awned floret in the cross cultivated × *A. fatua*. Partial linkage was observed between pubescence on lemma and brown color of lemma. He observed independent segregation between heavy pubescence on the lemmas of all florets of the wild oat and the yellow color of the lemmas of the cultivated oat.

Love and Fraser (*6*) crossed Burt and Red Rustproof (Red Texas) oats with *Avena fatua* and found a 1-factor difference for type of spikelet separation. The pointed-type base of *A. sativa* was dominant in F_1.

In a cross between *Avena fatua* (brown, hairy, awned) with Sixty-Day (yellow, glabrous, awnless), Love and Craig (*4*) also found the pointed base dominant in F_1 and the first floret of the spikelet often awned and pubescent. The second floret of the spikelet was never awned but was occasionally pubescent.

Coffman, Parker, and Quisenberry (*1*), in a study of variability in Burt, a cultivated variety of *Avena byzantina*, found two types of floret separation—the *sativa*, or articulate form, which separates by disarticulation, and the *byzantina*, or *sterilis*-like form, which separates by basifracture.

INHERITANCE OF AWNS

Love and Fraser (*6*), in a study of inheritance of awns in the cross Burt, a weak-awned variety, × *Avena fatua*, a strong-awned variety, found the F_1 nearly awnless. A few plants had awns on the first floret of some spikelets. All degrees of awning were observed in F_2. When grouped as awnless, partly awned, and fully awned (like *A. fatua*), a 1:2:1 ratio was obtained in a general way. Fully awned lines bred true in the F_3. Partially awned lines with few exceptions produced partially awned lines and fully awned lines in a ratio of 3:1. About

one-half of the lines classed as awnless produced awnless lines; the remainder segregated in the same manner as the partially awned lines.

Fraser (*3*), in a study of awns in the cross Burt × Sixty-Day, classified Burt as fully awned (first and second florets awned) and Sixty-Day as awnless. Both parents were constant pure-line cultures. There was "nearly complete dominance of the awnless condition." Awns segregated in F_2 in a ratio of approximately 1 awnless to 2 partly awned to 1 fully awned. In F_3, fully awned plants bred true and were recessive; nearly all partly awned plants produced a ratio of 3 not fully awned to 1 fully awned. A few of the partly awned plants produced awnless families. Awnless plants bred true. Spikelets having two awns each were found only on the plants which were 100 per cent awned. Fraser concluded that environmental factors affect awn production. Strong linkage was found between the fully awned condition (all spikelets having at least one awn) and the medium-long hairs on the base of the grain. Strong linkage also was found to exist between the fully awned condition and the nonarticulate type of floret separation in Burt.

INFLUENCE OF LEMMA COLOR ON AWN DEVELOPMENT

Nilsson-Ehle (*7*) found that varieties of oats with yellow grain have very much reduced awns in most cases. He concluded that the factor for yellow inhibits the production of awns.

Love and Craig (*4*) found a very definite relation between color of glumes and production of awns. Awn production appears to be independent of the genes for black and gray, but the gene for yellow inhibits their production.

The same authors (*5*) suggest that yellow fatuoids should be found in some yellow oats and not in others, since yellow inhibits awn development in some varieties.

Stanton and Coffman (*8*) observed yellow fatuoids in yellow-kerneled varieties such as Kherson, Richland, and Iogold.

PARENT MATERIAL USED IN CROSSES

The parents used in the crosses studied included forms and varieties of the following species: One of *Avena fatua*, two of *A. sterilis*, three of *A. sativa*, and two of *A. byzantina*. The cultivated varieties were pure lines that had been grown for several seasons by W. W. Mackie in the cereal nurseries at University Farm, Davis, Calif. The wild varieties also were pure lines that had been propagated from single plants. Two of these were obtained from Berkeley, Calif., and one from the Division of Cereal Crops and Diseases, United States Department of Agriculture, Washington, D. C.

The chromosome numbers of the species and varieties used in this experiment were not checked by the writer. However, several investigators have found that the number for *Avena fatua*, *A. sterilis*, and their cultivated derivatives is $21(n)$.[4]

The descriptions of the varieties used as parents deal chiefly with the characters studied.

[4] n=haploid chromosome number.

WILD FORMS

Avena Fatua

Avena fatua L., C. I.[5] 2795, is found growing wild in California. It was obtained by the late P. B. Kennedy, of the University of California, as a herbarium specimen from El Portal, Calif. It probably is more or less widely distributed in the State, although it is not so large or so vigorous as the varieties usually found in the interior valleys.

This *Avena fatua* is a short-straw early variety with spreading panicle. Spikelets 2 to 3-flowered, all florets dropping at maturity, with distinct oval pitted callus or scar at the base of each floret; the basal callus surrounded by ring of short stiff hair; kernels or florets small; lemmas brown and provided with strongly geniculate and twisted awns and dense brownish pubescence, especially on the two lower florets; the rachilla segment slender, with heavy pubescence on dorsal side (Pl. 1, C, F, and I); the upper node of the culm pubescent.

Avena Sterilis Macrocarpa

Plant selections of *Avena sterilis macrocarpa* Monsch., C. I. 2657, were collected by the writer on the campus of the University of California at Berkeley and grown as pure lines at Davis. It probably represents a type which had escaped from an early introduction. It has been found growing wild only on the campus and on adjoining vacant city lots.

Avena sterilis macrocarpa is the largest kerneled variety known of the *sterilis* oat group. It has moderately strong straw, a spreading panicle with large pendent spikelets; upper node of the culm pubescent; spikelets 2-flowered with one to three rudimentary florets; separation from the pedicel by abscission resulting in an oval pitted callus (distinct "sucker mouth") surrounded by a dense ring of hair; upper florets of the spikelet remain attached after maturity because of the heavy nonarticulate rachilla; lemmas yellowish white, the two lower florets provided with strongly geniculate and twisted awns and long white silky hair; the lower twisted portion of awns pubescent; the rachilla segments with dense medium-long hair on dorsal side. (Pl. 1, A, D, and G.)

Avena Sterilis Ludoviciana

Avena sterilis ludoviciana Durieu, C. I. 1780, was obtained from the Division of Cereal Crops and Diseases, United States Department of Agriculture, Washington, D. C. It has been considered as a transition form between the fully wild hairy species and the cultivated derivatives of *A. sterilis*.

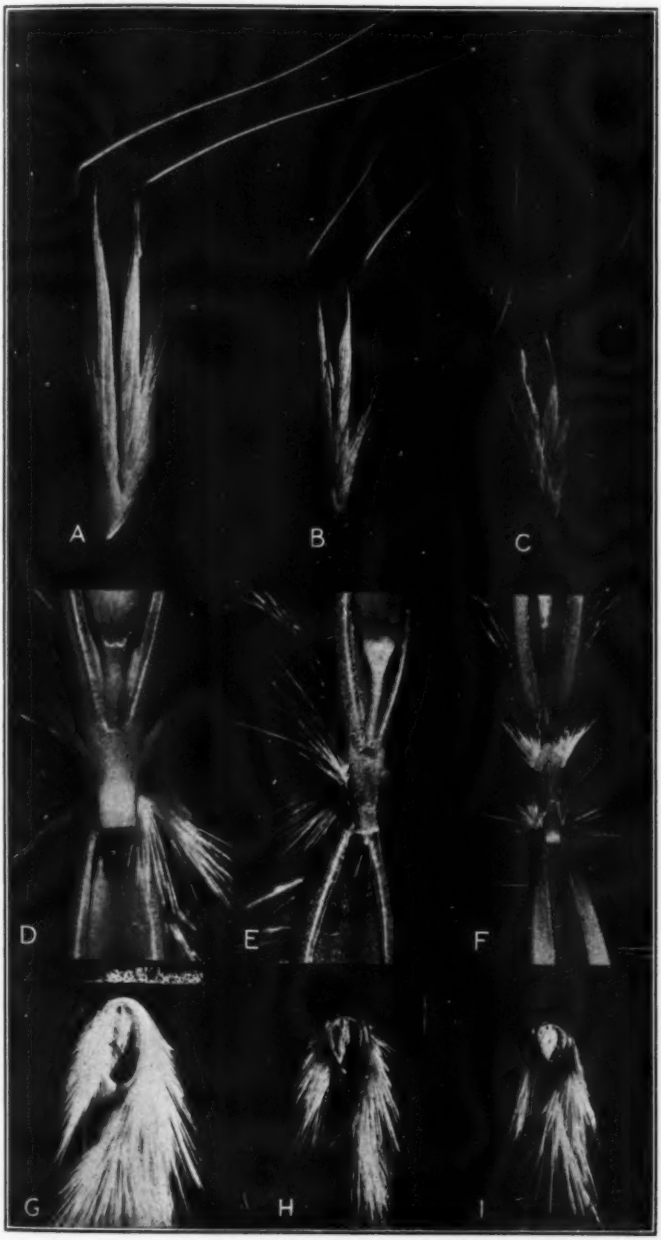
[5] C. I. refers to the accession number of the Division of Cereal Crops and Diseases, formerly Office of Cerial Investigations.

EXPLANATORY LEGEND FOR PLATE 1

A, B, C.—Spikelets. × 6. A, *A. sterilis macrocarpa;* B, F_1 hybrid; C, *A. fatua.* F_1 spikelet (B) intermediate in size but nearer that of *fatua* (C).

D, E, F.—Types of attachment of second floret to its rachilla. × 15. D, *A. sterilis macrocarpa;* E, F_1 hybrid; F, *A. fatua.* Solidified attachment of *A. sterilis macrocarpa* (D) dominant in F_1 (E). Second floret turned vertically, above oppositely turned lower floret. (Rachilla attachment prepared for photographing by pulling apart two lower florets, after soaking in water to prevent fracture or disarticulation of rachilla.) Type of attachment visible in green material through ordinary hand lens.

G, H, I.—Types of spikelet separation. × 15. G, *A. sterilis macrocarpa;* H, F_1 hybrid; I, *A. fatua.* Callus, or scar, of F_1 hybrid (H) similar to that of parents (G and I), but intermediate in size and shape.



Avena fatua × *A. sterilis macrocarpa*: Parents and F_1 hybrid

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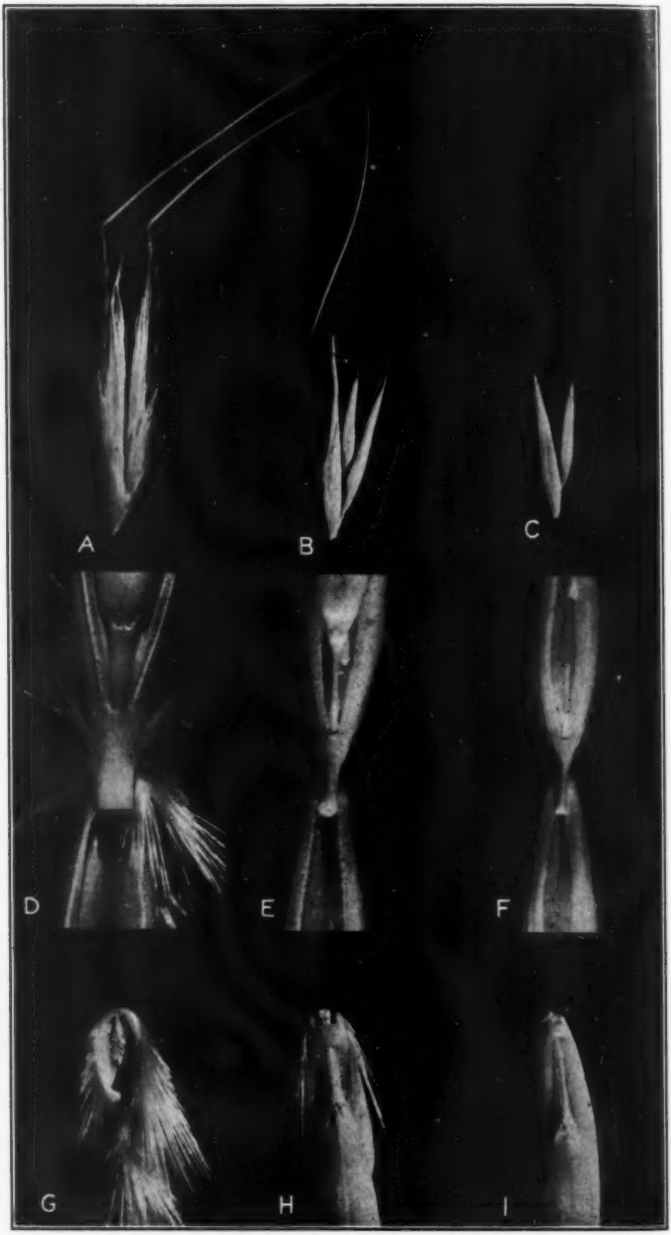


Avena sterilis macrocarpa × *A. sativa* var. Richland: Parents and F_1 hybrid

For explanatory legend see opposite page

It has mid-sized glabrous lemmas, nonpubescent lower awns, and glabrous culm nodes. In other characters it is very similar to *Avena sterilis macrocarpa*.

CULTIVATED FORMS

AVENA SATIVA

In varieties of the cultivated oat, *Avena sativa* L., spikelet and floret separation occur by fracture and disarticulation, respectively. The lemma usually is glabrous, but occasionally a few scattered hairs are found on the lemma of the first floret. Awns usually are neither twisted nor geniculate. They vary from weak to very weak and may be entirely wanting except for an occasional awn. A light tuft of short hair persists at the base of the first floret in some varieties, but in most of them it is completely wanting except for an occasional short hair. The rachilla segment of the second floret is slender and ranges from 1 mm. to about 3 mm. in length. The small scar ("sucker mouth") at the upper end of the rachilla segment, which is so well defined in its ancestor, *A. fatua*, is poorly defined or wanting in *A. sativa*.

RICHLAND, C. I. 787.—This is a short-straw early variety with a rather small spreading panicle. Spikelets usually are 2-flowered; florets are small, yellow, glabrous, and generally awnless except for an occasional panicle having a few weak awns. Spikelet separation occurs by fracture with callus scar obscure or not evident; floret separation from its rachilla segment occurs by disarticulation. (Pl. 2, C, F, and I.)

NORTH FINNISH, C. I. 1801.—North Finnish is an early to mid-season strong-straw variety with erect spreading panicles whose branches are more or less ascending. Spikelets are 2 to 3 flowered, usually 2-flowered, florets are mid-sized, brownish black, glabrous, awnless except for a few (one to six) weak awns per panicle; spikelet and floret separation similar to that in Richland; rachilla segment slender and from 2 to 3 mm. long.

AWNLESS PROBSTEIER, C. I. 1888.—This is a strong-straw late-maturing variety with spreading panicles. Spikelets 2 to 3 flowered; florets mid-sized, short, plump, glabrous, yellow, awnless except, rarely, one to two very weak awns per panicle; short basal hairs (1 to 2 mm.) sometimes present; spikelet and floret separation similar to that in Richland; rachilla segment of the second floret short and slender.

AVENA BYZANTINA

The spikelet characters found in the cultivated derivative of *Avena sterilis* L., designated as *A. byzantina* by C. Koch, have been modified from those of their wild ancestor in a manner similar to those in *A. sativa*. The main difference is found in the presence of a much reduced but still persistent rachilla segment of the second floret of

EXPLANATORY LEGEND FOR PLATE 2

A, B, C.—Spikelets. × 6. A, *A. sterilis macrocarpa;* B, F_1 hybrid; C, Richland. F_1 spikelet (B) hairy and geniculately awned only on lemma of first floret, thus resembling both parents (A and C).

D, E, F.—Types of attachment of second floret to its rachilla. × 15. D, *A. sterilis macrocarpa;* E, F_1 hybrid; F, Richland. Attachment of slender rachilla of Richland (F) seen as slight notch or depression (scarcely visible in picture). *Sterilis* type of attachment (D) dominant in F_1 (E), but size nearer that of *sativa* (Richland) parent (F).

G, H, I.—Types of spikelet separation. × 15. G, *A. sterilis macrocarpa;* H, F_1 hybrid; I, Richland. Pointed base of spikelet separation of *sativa* (Richland) parent (I) almost completely dominant in F_1 hybrid (H).

the spikelet which, when separation occurs at maturity, is fractured at the base. The rachilla segment is variable in length in both species but often is more slender in varieties of *A. sativa*.

FULGHUM, C. I. 708.—Fulghum is a mid-tall stiff-straw variety with spreading panicle whose branches are more or less ascending. Spikelets usually 3-flowered; florets mid-sized, plump, reddish in color, glabrous, awnless, but occasional plants are found with one to three weak awns per panicle; a few basal hairs usually present; the rachilla segment on the second floret moderately strong and persistent or nonarticulate. (Pl. 3, C, F, and I.)

COASTBLACK, C. I. 1025.—Coastblack is a strong-straw, very late-maturing variety with spreading panicle. Spikelets from 2 to 3 flowered; florets large; lemmas glabrous or with a few scattered hairs on lemma of first floret; moderately long, weak, and nontwisted awns usually present on both lower florets; a light tuft of long hair at base of first floret; the rachilla segment on the second floret moderately strong and persistent. (Pl. 4, C, F, and I.)

CHARACTERS STUDIED

The study centered mainly on the inheritance of types of rachilla attachment and separation of the second floret and the associated species-type characters—separation of spikelet, degrees of awning, and pubescence of rachilla and callus. A study also was made in certain crosses of the inheritance of the characters hairiness of lemmas, color of grains, and pubescence of culm nodes.

An oat spikelet consists of 2 to 5 or 6 florets. In the cultivated varieties usually from 2 to 3 florets develop into kernels. The upper florets remain rudimentary. In the wild species also from 2 to 3 kernels are formed. In this paper specific florets are designated first, second, third, etc., beginning with the lowest, or the one attached to the peduncle.

The rachilla segment of the second floret and the mode of its separation from the first floret of the spikelet were studied in all crosses. In *Avena fatua* the upper end of the slender rachilla segment, which articulates with the floret it carries, is seen as a spatulate termination distinctly visible under an ordinary hand lens. (Pl. 1, F, and pl. 5, A, lower lemma.) The florets or kernels of the spikelet fall apart at maturity, forming an oval pitted callus at the lower end of each lemma. (Pl. 5, A.) In *A. sterilis* the rachilla segment of the second floret is nonarticulate, separation occurs only by fracture (basifracture) (*1*) and there is no evidence of a callus or scar on the base of the lemma. (Pl. 5, C, upper lemma.) At maturity the spikelet as a whole falls to the ground as the result of the separation (disarticulation) of the first floret from the pedicel. (Pl. 5, C, lower lemma.)

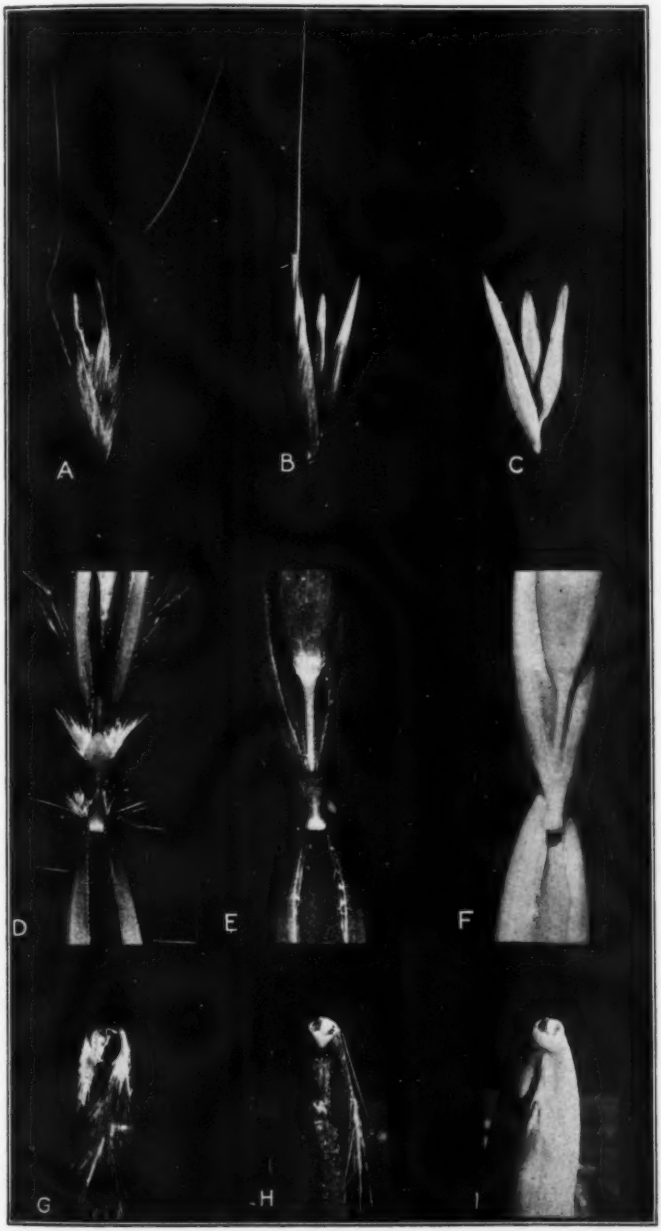
In both wild species the awn is sharply bent, or geniculate, near its middle. The strands of the awn are twisted in a clockwise direc-

EXPLANATORY LEGEND FOR PLATE 3

A, B, C.—Spikelets. × 6. A, *A. fatua;* B, F_1 hybrid; C, Fulghum. F_1 spikelet (B) hairy, and awned only on first floret. Awn rather weak, frequently without geniculation, and only slightly twisted.

D, E, F.—Types of attachment of second floret to its rachilla. × 15. D, *A. fatua;* E, F_1 hybrid; F, Fulghum. *Sterilis* type of rachilla attachment of Fulghum (F) apparently completely dominant over *fatua* type (D) in F_1 (E). Short rachilla of Fulghum also dominant in F_1.

G, H, I.—Types of spikelet separation. × 15. G, *A. fatua;* H, F_1 hybrid; I, Fulghum. Cultivated base of spikelet separation of Fulghum (I) dominant in F_1 (H) over wild base of *fatua* (G).



Avena fatua × *A. byzantina* var. Fulghum: Parents and F_1 hybrid

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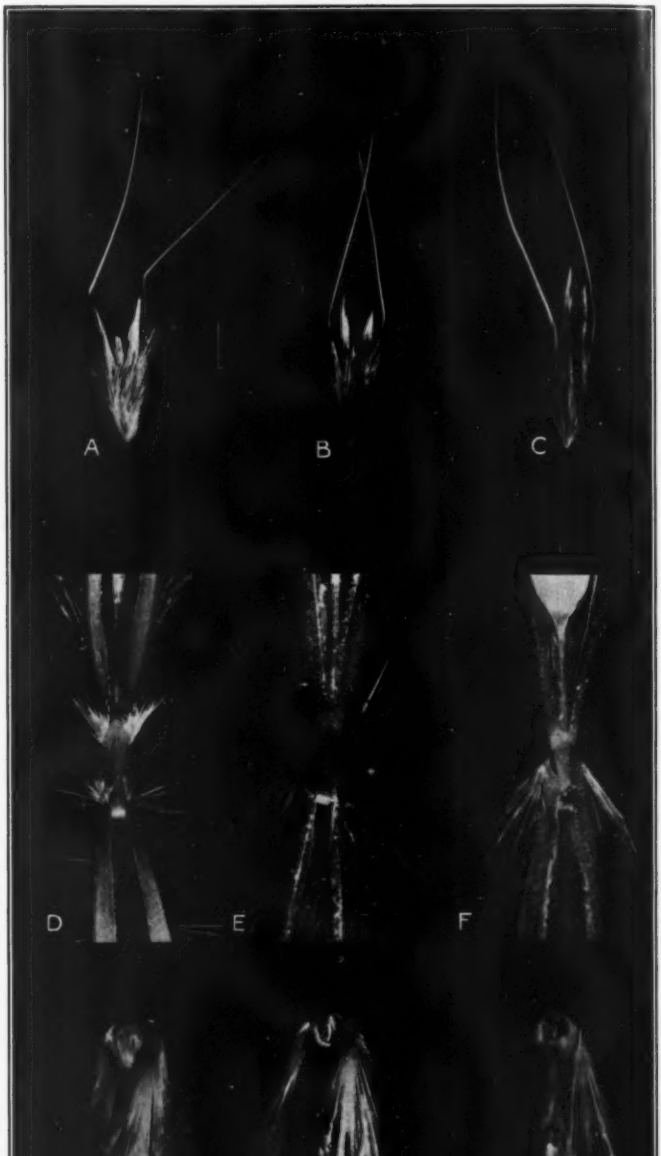


Avena fatua × *A. byzantina* var. Coastblack: Parents and F_1 hybrid

For explanatory legend see opposite page

tion below the geniculation. The geniculation and twisting are frequently absent in the awns of cultivated varieties.

In hairy varieties the long to moderately long hairs are distributed over the lower part of the lemma from its base to near or slightly above the point of attachment of the awn. The ring of short stiff hairs surrounding the callus occurs independently of the hair on the lemma.

The color of grain refers to the color of the lemma and palea, but principally to the lemma.

The term "pubescent node," used in this paper, refers to a zone (1 to 2 mm. wide) of more or less scattered hairs found just above and below the upper node of the culm in the two wild species.

METHODS

The seeds of parents and hybrids were space planted in nursery rows 1 foot apart. The rows of *Avena fatua* and *A. sterilis* and those of their hybrids were harvested into separate envelopes at early maturity to prevent total loss and mixing of seed due to the deciduous habit of these forms. Other plants were pulled at maturity and tied into bundles. One panicle of each plant of F_3 nonshattering families was harvested and placed in a single large envelope. Seeds of the wild-type plants were separated and the awns removed by hand where necessary in preparation for seeding.

EXPERIMENTAL DATA

Data are presented on the following crosses: *Avena fatua*×*A. sterilis macrocarpa; A. fatua*×*A. sterilis ludoviciana; A. fatua*×*A. byzantina* var. Coastblack; *A. fatua*×*A. byzantina* var. Fulghum; *A. sterilis ludoviciana*×*A. sativa* var. Probsteier; *A. sterilis ludoviciana*×*A. sativa* var. North Finnish; *A. sterilis macrocarpa*×*A. sativa* var. Richland.

The usual precautions were observed in making the crosses. The flowers were carefully emasculated and covered with glassine bags. Pollinations were made from the same day up to six days after emasculation. Mature or nearly mature anther sacs were collected with a forceps and placed in U-shaped clean paper containers. Anthesis took place at approximately 4 o'clock every afternoon during the flowering period. Pollen was collected shortly before or at anthesis and applied to the stigmas of the emasculated flowers as quickly as possible.

A list of oat crosses and reciprocals, with the number of flowers pollinated, kernels harvested, and percentage of successful crosses, is given in Table 1. Crosses were obtained without great difficulty. The largest number of successful pollinations were obtained from

EXPLANATORY LEGEND FOR PLATE 4

A, B, C.—Spikelets. ×4. A, *A. fatua;* B, F_1 hybrid; C, Coastblack. F_1 spikelet (B)—F_2 plant figured segregating in F_2 in the same manner as in F_2—geniculately awned and hairy on both florets, but with these characters weaker than in *fatua*. Note well-developed awns in Coastblack (C).

D, E, F.—Types of attachment of second floret to its rachilla. ×15. D, *A. fatua;* E, F_1 hybrid; F, Coastblack. Coastblack (F) found to contain two independent pairs of factors for *sterilis* type of rachilla, one probably associated with *byzantina* complex, the other with wild *sterilis* complex. *Sterilis* type of rachilla attachment of Coastblack (F) dominant in F_1 (E).

G, H, I.—Types of spikelet separation. ×15. G, *A. fatua;* H, F_1 hybrid; I, Coastblack. Callus, or scar, of F_1 hybrid (H) moderately well-developed, as that of Coastblack (I).

crosses of *Avena fatua* with both *A. sterilis* and *A. byzantina* when *A. fatua* was used as the female parent.

TABLE 1.—*Interspecific oat crosses and reciprocals and number of heads and flowers pollinated, kernels obtained, and percentage of successful crosses, 1925*

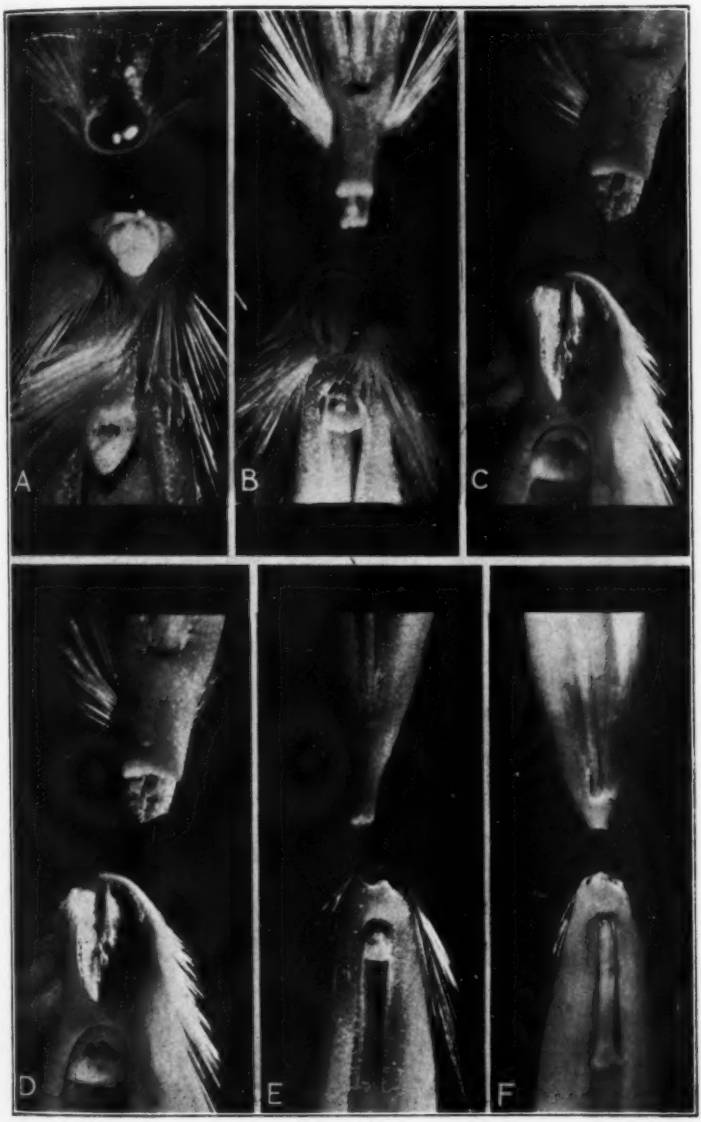
Cross	Panicles used	Florets pollinated	Kernels obtained	Successful crosses
	Number	*Number*	*Number*	*Per cent*
Avena fatua×A. sterilis macrocarpa	2	38	27	71.1
Reciprocal	2	44	16	36.4
A. fatua×A. sterilis ludoviciana	3	72	29	40.3
Do	1	14	0	0
Reciprocal	2	92	14	15.2
A. byzantina var. Coastblack×A. fatua	2	94	6	6.4
Reciprocal	2	52	6	11.5
A. byzantina var. Fulghum×A. fatua	2	34	10	29.4
Reciprocal	1	24	16	66.7
A. sativa var. Probsteier×A. sterilis ludoviciana	1	62	3	4.8
Do	1	62	0	0
Reciprocal	2	64	30	46.9
A. sativa var. North Finnish×A. sterilis ludoviciana	2	90	19	21.1
Reciprocal	1	40	5	12.5
Do	1	40	0	0
A. sterilis macrocarpa×A. sativa var. Richland	2	24	0	0
Reciprocal	2	58	19	32.8

F_1 HYBRIDS

A comparative description of the type of floret separation, character of awns, hairiness of lemma and hairiness of rachilla segment, color of lemma (in seven crosses), and pubescence of culm nodes (in one cross) in the F_1 progeny and in the parents, is given in Table 2.

EXPLANATORY LEGEND FOR PLATE 5

A.—*A. fatua;* B, *A. fatua* × *A. sterilis macrocarpa*, F_1; C and D, *A. sterilis macrocarpa;* E, *A. sterilis macrocarpa* × Richland, F_1; F, Richland. *Sterilis* type of floret separation (by basifracture) occurs in F_1 of both crosses (B and E). In *sterilis* form (C or D), and usually in F_1 hybrids, rachilla remains attached to second floret and break occurs at base of rachilla where it is attached to first floret. In *fatua* form (A) separation always occurs by disarticulation (abscission), and in *sativa* form (F) separation usually occurs by disarticulation (semiabscission), leaving entire rachilla attached to lower floret.



Avena fatua × *A. sterilis macrocarpa* and *A. sterilis macrocarpa* × *A. sativa* var. Richland: Types of floret separation in parents and F_1 hybrids when first and second florets are pulled apart. × 15

For explanatory legend see opposite page

Avena fatua × *A. byzantina* var. Fulghum and *A. fatua* × *A. byzantina* var. Coastblack. Types of floret separation in parents and F_1 hybrids when first and second florets are pulled apart. × 15

For explanatory legend see opposite page

Table 2 shows that the nonarticulate type of floret separation characteristic of both *Avena sterilis* and *A. byzantina* is dominant over the articulate type of floret separation in *A. fatua* and *A. sativa*. (Pl. 5, B, and Pl. 6, B and E.) It was found, but not shown in Table 2, that separation by fracture of the spikelet from its pedicel, as in *Avena sativa*, is almost completely dominant over separation of the spikelet by abscission, as in both *Avena fatua* and *A. sterilis*. (Pl. 2, H.)

Medium strongly geniculate and twisted awns and intermediate hairy lemmas occur on both the first and second florets in F_1 of *Avena atua* × Coastblack or its reciprocal (Pl. 4, B), but hair is absent on the rachilla segment of the second floret. (Pl. 4, E.) In crosses of *A. fatua* and *A. sterilis* (awns strongly geniculate and twisted) with

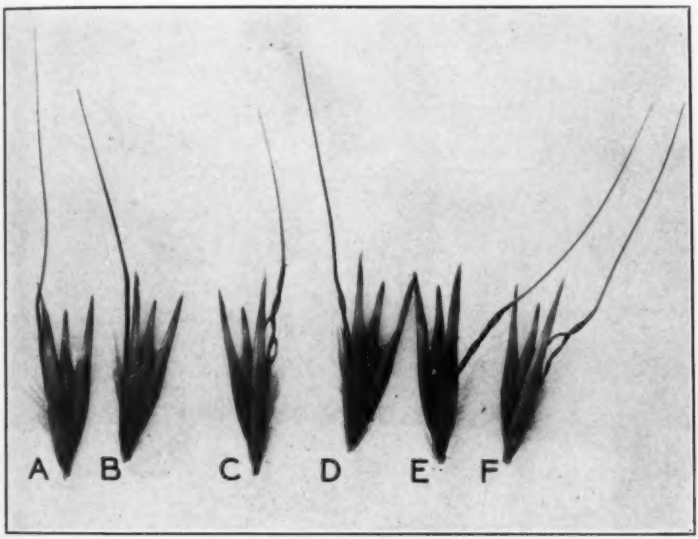


FIGURE 1.—F_1 oat spikelets of the cross *Avena sterilis macrocarpa* × *A. sativa* var. Richland, showing normal awns (A and B) and untwisting awns (C, D, E, and F)

the cultivated varieties (awns absent or weak), medium strongly geniculate and twisted awns are found only on the first floret of F_1 plants. (Pl. 2, B, and Pl. 3, B.)

The twisted lower portion of the awn was untwisted into loops in many F_1 plants of the cross *Avena sterilis macrocarpa* × Richland and occasionally in other crosses. (Fig. 1.) This condition also was found in heterozygotes of both F_2 and F_3.

In *Avena fatua* (hairy) × Fulghum (glabrous) the first floret was medium hairy, while the second was glabrous or occasionally had a

EXPLANATORY LEGEND FOR PLATE 6

A.—*A. fatua;* B, *A. fatua* × Fulghum, F_1; C, Fulghum; D, *A. fatua;* E, *A. fatua* × Coastblack, F_1; F, Coastblack. *Sterilis* type of floret separation (by basifracture) occurs in F_1 of both crosses (B and E). In all F_1 hybrids, as in *byzantina* forms (C and F), rachilla remains attached to second floret and break occurs at base of rachilla where it is attached to first floret. In *fatua* form (A or D) separation always occurs by disarticulation (abscission).

TABLE 2.—*Characters of parent and F_1 hybrid oat plants*

Variety or hybrid	Type of floret separation	Characters of awns of first and second florets	Hairiness of lemma on first and second florets	Hairiness of rachilla segment of second floret	Color of lemma	Pubescence of culm nodes
Parent						
Avena fatua	Articulate [a]	Strongly geniculate and twisted	Heavy	Hairy	Brown	Pubescent.
A. sterilis ludoviciana	Nonarticulate [b]	do	Glabrous	do	Grayish white	
A. sterilis macrocarpa	do	do	Long and heavy	do	Yellowish white	Do.
A. byzantina var. Coastblack	do	Weak; nontwisted	Glabrous; sometimes few hairs on first.	Glabrous	Black	
A. byzantina var. Fulghum	do	Absent or very weak on one or both.	Glabrous	do	Reddish	
A. sativa var. Probsteier	Articulate	Absent or sometimes very weak on first.	do	do	White	
A. sativa var. North Finnish	do	Absent; 1–6 weak in panicle	do	do	Black	
A. sativa var. Richland	do	Absent; sometimes 1–2 weak in panicle.	do	do	Yellow	Glabrous.
F_1 Hybrid						
A. fatua × A. sterilis ludoviciana	Nonarticulate	Medium strongly geniculate and twisted.	Heavy	Hairy	Brown	
A. fatua × A. sterilis macrocarpa	do	do	do	do	do	
A. fatua × A. byzantina var. Fulghum	do	do	Medium heavy on first.	Glabrous	Light brown	
A. byzantina var. Coastblack × A. fatua	do	do	Medium heavy	do	Black	
A. sterilis ludoviciana × A. sativa var. Probsteier.	Nonarticulate (intermediate).	Strongly geniculate and twisted.	Glabrous	do	White	
A. sterilis ludoviciana × A. sativa var. North Finnish.	do	do	do	do	Brown	
A. sterilis macrocarpa × A. sativa var. Richland	do	do	Hairy on first	do	Yellowish white	Pubescent.

[a] Separation by disarticulation as described by Coffman, Parker, and Quisenberry (*1*).
[b] Separation by basifracture as described by Coffman, Parker, and Quisenberry (*1*).

few scattered hairs. In *A. sterilis macrocarpa* (hairy) × Richland (glabrous) only the first floret was hairy.

Brown color of lemma was dominant over white or light colored, and black over brown.

Culm nodes were pubescent in the F_1 of *Avena sterilis macrocarpa* (hairy) × Richland (glabrous).

F_2 HYBRIDS

Floret Separation

Type of floret separation was studied in F_2 in all seven crosses. Three classes were recognized, based on thes pecies crossed, *fatua–sterilis* (two crosses), *fatua–byzantina* (two crosses), and *sterilis–sativa* (three crosses). (Table 3.)

Table 3.—*Segregation in F_2 for type of floret separation, and other characters in seven interspecific crosses in oats*

Class and cross	Type of floret separation [a]					Deviation	P. E.	Dev./P. E.	Total plants
	Nonarticulate [b]			Articulate [c]					
	Byzantina	Sterilis	Intermediate	Sativa	Fatua				
Fatua-sterilis:									
Avena fatua×A. sterilis ludoviciana—									*Number*
Observed		335			95				430
Calculated (3:1)		322.5			107.5	12.5	6.06	2.06	
A. fatua × A. sterilis macrocarpa—									
Observed		235			68				303
Calculated (3:1)		227.3			75.8	7.8	5.08	1.5	
Fatua-byzantina:									
A. fatua×Fulghum—									
Observed	331	[d] 4		3	140				478
Calculated (3:1)	353.2				117.8	22.2	6.34	3.5	471
Coastblack × A. fatua—									
Observed	295	85			28				408
Calculated (12:3:1)	306	76.5			25.5				
Deviation (χ^2=1.590; P=0.466)	−11	+8.5			+2.5				
Sterilis-sativa:									
A. sterilis ludoviciana × Probsteier—									
Observed		193	301	214					708
Calculated (1:2:1)		177	354	177					
Deviation (χ^2=17.1158; P=0.0002)		+16	−53	+37					
A. sterilis ludoviciana × North Finnish—									
Observed		368	720	300					1,388
Calculated (1:2:1)		347	694	347					
Deviation (χ^2=8.6138; P=0.0139)		+21	+26	−47					
Richland × A. sterilis macrocarpa—									
Observed		82	137	77					296
Calculated (1:2:1)		74	148	74					
Deviation (χ^2=1.8040; P=0.4047)		+8	−11	+3					

[a] Other species characters include: For *byzantina*, cultivated base and nonarticulate florets; for *sterilis*, strongly geniculate and twisted awns on first and second florets, pitted callus surrounded by ring of hairs at base of lemma of first floret, and hairy rachilla; for intermediate, floret separation as it occurs in heterozygotes; for *sativa*, cultivated base and articulate second floret; and for *fatua*, strongly geniculate and twisted awns on first and second floret, pitted callus surrounded by ring of hairs on the base of all lemmas and hairy rachilla.

[b] Separation by basifracture occurs as described by Coffman, Parker, and Quisenberry (*1*).

[c] Separation by disarticulation occurs, as described by Coffman, Parker, and Quisenberry (*1*) for *sativa* and by abscission for *fatua*.

[d] Crossover plants.

Segregation for type of floret separation of approximately 3 nonarticulate (*sterilis*) to 1 articulate (*fatua*) was obtained in the *fatua-sterilis* crosses. Both crosses had deviations from the calculated well within the usual limit of three times the probable error, indicating a 1-factor difference for this character.

A segregation of approximately 3 nonarticulate (*byzantina*) to 1 articulate (*fatua*) was obtained in *Avena fatua* × Fulghum, but the deviation was slightly greater than three times the probable error (3.5). A part of the deviation from the normal 3:1 ratio no doubt resulted from the occurrence of unexpected types of plants. Four wild *sterilis* plants were found in a population of 478 individuals. The possibility was not overlooked that these *sterilis* plants might have represented mechanical mixtures, and therefore an F_1 population from seed of the same cross was grown in 1930 at Arlington Experiment Farm, near Washington, D. C. Wild *Avena sterilis* is known to be absent in this locality, and none was grown at Arlington farm this year. However, six wild *sterilis* type plants were found in the F_2 progeny consisting of 974 individuals. In the first-mentioned population three plants having the *A. sativa* type of floret separation were found; in the second, four such plants were found. Four of these plants had glabrous lemmas and weak awns (slightly geniculate in one) on the first floret; the remaining plants were hairy and medium strongly awned on the first floret. Light hairiness of the rachilla of the second floret was present in some of these plants. This behavior indicates that type of floret separation and expression of awns and associated characters are controlled by a number of closely linked factors, as suggested by Surface (*9*), and that crossing over occurred between the factor that conditions type of floret separation and the remaining factor complex. The percentage of crossing over for the 478 F_2 plants grown at Davis, Calif., was 1.46 and that for all F_2 plants, or 1,452 individuals, 1.17.

In the F_2 of *Avena fatua* × Coastblack, 380 plants with florets separating by fracture (*byzantina* and *sterilis*) to 28 articulate (*fatua*) separating by abscission were obtained. This gives a ratio very close to 15 nonarticulate to 1 articulate (14.902 to 1.098), which indicates a 2-factor difference for type of floret separation. Based on the oat types usually recognized there were 295 *byzantina*, 85 *sterilis*, and 28 *fatua* plants. This segregation gives a very good fit to the ratio of 12 *byzantina* to 3 *sterilis* to 1 *fatua* ($P = 0.466$). While the type of floret separation is the same for both *byzantina* and *sterilis*, yet these two oat forms can be separated readily by the presence in *sterilis* of strongly geniculate and twisted awns on both first and second florets, the pitted oval spikelet callus with associated ring of hairs, and the hairy rachilla segments. (Fig. 2.) The recombination resulting in the *sterilis* type will be discussed later.

In the three *sterilis–sativa* crosses, observed numbers giving ratios fairly close to 3 *sativa* and intermediate to 1 *sterilis* were obtained, indicating a single-factor difference for species type in all. All crosses had deviations well within the limits of three times the probable error. The *sterilis* segregates could be readily distinguished, but some difficulty was encountered in accurately separating the *sativa* from the intermediate segregates. The last two were classified by their type of floret separation. Florets of intermediates separated by basifracture or heterofracture of the rachilla segment; florets of *sativa* types sep-

arated by disarticulation at the place of rachilla-segment attachment. Calculated on a basis of 1 *sterilis* to 2 intermediate to 1 *sativa*, values of *P* were 0.0002 for *Avena sterilis ludoviciana* × Probsteier, 0.0139 for *A. sterilis ludoviciana* × North Finnish, and 0.4047 for Richland × *A. sterilis macrocarpa*. The wide deviations and low probabilities obtained in the first two crosses may be attributed partly to slight immaturity of some plants.

Inheritance of awns was followed in detail in several crosses. Occasional intermediate nonarticulate awnless plants and intermediate articulate awned plants (only first floret strongly awned) were found. Variations in expression of the awnless or weak-awned condition were observed, but no definite segregating ratio was found other than that indicated as 1 (awnless and partly awned) to 2 (first floret more or

FIGURE 2.—Parents (three spikelets of each) and F_2 segregates (three spikelets of each) of the cross *Avena fatua* × *A. byzantina* var. Coastblack arranged in groups. Parents: A, *A. fatua;* B, Coastblack. F_2 segregates: C, *fatua;* D and E, *byzantina;* F and G, intermediate; H, wild *sterilis*

less strongly awned) to 1 (strongly geniculate and twisted awns on both florets). All *fatua* and *sterilis* plants had strongly geniculate and twisted awns on both lower florets; in fact, the wild-oat complex of characters was always accompanied by them. So far as this experiment is concerned it may be said that in all crosses, with the exception of the crossing over noted in the cross *avena fatua* × Fulghum, there was complete linkage between the factor for strongly geniculate and twisted awns and for spikelet separation by abscission.

HAIRINESS OF LEMMA

Both first and second lemmas were hairy in the F_1 of the cross *Avena fatua* (hairy lemmas) × *A. sterilis ludoviciana* (glabrous lemmas). In an F_2 family of 430 plants, 313 were hairy and 117 were glabrous. This is close to a 3:1 ratio, indicating a 1-factor difference for hairiness of lemma. The deviation from the calculated was 1.6 times the probable error.

Hairiness of lemma was studied in the F_2 of two additional crosses. In the F_1 of *Avena fatua* × Fulghum and of *A. sterilis macrocarpa* × Richland only the first lemma was hairy. A ratio of approximately 1 (both hairy) to 2 (only first hairy) to 1 (both glabrous) was obtained

in the F_2 of the first-named cross, which suggests a 1-factor difference for hairiness of lemma. In the second cross, Richland × *A. sterilis macrocarpa*, the segregation for hairy lemmas in an F_2 family of 504 individuals was 61 (both hairy), 310 (only first hairy), and 133 glabrous, or a ratio of approximately 2:10:4. The character expression of the hairy classes had been altered, presumably by an inhibitor.

Color of Lemma

Color of lemma was studied in the cross *Avena fatua* (brown) × *A. sterilis ludoviciana* (grayish white). The color in F_1 was a lighter brown than that of the brown parent. Dark browns and light browns were observed in F_2 but in some cases the two classes of browns could not be distinguished with certainty. There were 314 brown or brownish and 116 grayish-white individuals in a family of 430 plants with an observed ratio of 2.92:1.08. The deviation from the calculated was 1.4 times the probable error.

Pubescence of Nodes

The inheritance of pubescence of culm nodes was studied in the cross Richland (glabrous nodes) × *Avena sterilis macrocarpa* (pubescent upper culm node). The nodes were classed as pubescent in F_1. Variation in development of the hairy-node character was studied in the F_2. Some plants had well-developed pubescence on the upper node, while in others pubescence occurred only on the upper half of the node. The extent of hairiness ranged from moderately heavy to only a few scattering hairs. Plants having only a few scattering hairs also were classed as hairy. In a family of 296 plants, 222 had hairy upper nodes and 70 glabrous nodes. The deviation from the theoretical 3:1 ratio was only 0.8 of the probable error. This indicates a 1-factor difference for pubescent upper node. Owing to wide discrepancies in ratios, the inheritance of this character in F_3 could not be definitely verified.

F_3 HYBRIDS

Floret Separation

Space permits a presentation of only a summary of the data on segregations recorded in F_3 by individual families. Data on segregation by groups of families for the crosses studied are shown in Table 4. The findings on the inheritance of type of floret separation in F_3 are in agreement with the results obtained in the F_2 in all crosses. Besides true-breeding families, segregations of approximately 3 separating by fracture to 1 by disarticulation were obtained in seven crosses, showing that type of floret separation was conditioned by a 1-factor difference in these combinations. Segregating families of the two crosses *Avena fatua* × *A. sterilis* produced a ratio of close to 3 *sterilis* type to 1 *fatua* type, and the cross *A. fatua* × Fulghum, 3 *byzantina* type to 1 *fatua* type of floret separation. In the cross *A. fatua* × Fulghum the only one of the *sterilis* type plants from the F_2 from which progeny was obtained produced a true-breeding (constant) *A. sterilis* family of plants in F_3. This is positive evidence against natural crossing in the F_1 generation as being responsible for the occurrence of *sterilis* type plants in this cross. If natural crossing had occurred between F_1 plants and *sterilis* plants there would have been segregation for *fatua* and *sterilis* plants in F_3.

TABLE 4.—*F_3 breeding behavior for inheritance of type of floret separation and species types in seven interspecific crosses of oats*

Cross and F_2 type sown	Number of F_3 families	Type of floret separation and observed segregation	Calculated ratio	Dev. P. E.	Total number of plants
Avena fatua × A. sterilis ludoviciana:					
Nonarticulate(sterilis)	2	Homozygous sterilis			58
Do	12	467 sterilis to 166 fatua	3:1	1.05	633
Articulate (fatua)	6	Homozygous fatua			264
Total	20				955
A. fatua × A. sterilis macrocarpa:					
Nonarticulate (sterilis)	6	Homozygous sterilis			138
Do	9	164 sterilis to 51 fatua	3:1	0.64	215
Articulate (fatua)	4	Homozygous fatua			168
Total	19				521
A. fatua × Fulghum:					
Nonarticulate (byzantina and sterilis)—					
Byzantina	8	Homozygous byzantina			457
Do	26	1,120 byzantina to 386 fatua (and 5 sterilis).[a]	3:1	0.26	1,511
Sterilis	1	Homozygous sterilis			24
Articulate—					
Fatua	5	Homozygous fatua			310
Total	40				2,302
Coastblack × A. fatua:					
Nonarticulate (byzantina and sterilis)—					
Byzantina	20	Homozygous byzantina			869
Do	2	68 byzantina to 20 sterilis	3:1	0.73	88
Do	11	402 byzantina to 99 sterilis to 41 fatua (χ^2=1.61; P=0.462).	12:3:1		542
Do	1	41 byzantina to 11 fatua	3:1	.95	52
Sterilis	3	Homozygous sterilis			91
Do	13	420 sterilis to 136 fatua	3:1	.44	556
Articulate—					
Fatua	7	Homozygous fatua			379
Total	57				2,577
North Finnish × A. sterilis ludoviciana:					
Articulate and nonarticulate (sativa and intermediate)	10	Homozygous sativa			535
Do	6	do.[b]			256
Do	10	132 sativa[c] to 318 intermediate[d] to 148 sterilis (χ^2=3.2710; P=0.1993).	1:2:1		598
Nonarticulate (sterilis)	6	Homozygous sterilis			72
Total	32				1,461
A. sterilis ludoviciana × Probsteier:					
	11	Homozygous sativa			497
	10	179 sativa[c] to 320 intermediate to 143 sterilis (χ^2=4.0436; P=0.1330.	1:2:1		642
Nonarticulate (sterilis)	5	Homozygous sterilis			50
Total	26				1,189
Richland × A. sterilis macrocarpa:					
Articulate and nonarticulate (sativa and intermediate)	11	Homozygous sativa			389
Do	11	356 sativa and intermediate to 105 sterilis.	3:1	1.63	461
Nonarticulate (sterilis)	4				32
Total	26				882

[a] The crossover *sterilis* plants not included in the calculation for goodness of fit. Expected crossover plants having *A. sativa*-like floret separation, if any present, were included with *byzantina*.
[b] 2 to 10 per cent of plants having strongly geniculate awns on first floret.
[c] First floret awnless or weak awned.
[d] Including a few plants classed as awnless.

Heterozygous families of the crosses North Finnish × *Avena sterilis ludoviciana* and *A. sterilis ludoviciana* × Probsteier segregated in ratios of approximately 1 *sterilis* type to 2 intermediate type to 1 *sativa* type of floret segregation. Values of *P* indicated a satisfactory fit in both crosses. Awnless plants having the intermediate type of floret separation, and intermediate-awned plants (first floret strongly awned) having the *sativa* type were observed in several segregating families of both crosses.

The findings in the F_3 of the cross Coastblack × *Avena fatua* support the evidence for a 2-factor difference for type of floret separation suggested by the segregation in F_2 of approximately 12 nonarticulate (*byzantina*) to 3 nonarticulate (*sterilis*) to 1 articulate (*fatua*). F_2 plants with florets separating by fracture (*byzantina*) produced four types of families as follows: (1) Homozygous *byzantina;* (2) segregating into 3 *byzantina* to 1 *sterilis;* (3) segregating into 3 *byzantina* to 1 *fatua;* and (4) segregating into 12 *byzantina* to 3 *sterilis* to 1 *fatua.* Plants having all the characters of the "*sterilis* character complex" produced some homozygous *sterilis* families and others that segregated into 3 *sterilis* to 1 *fatua.* *Fatua* plants yielded only *fatua* families. The F_2 classifications apparently were made correctly, as there was no overlapping of classes. The segregations found and the new *sterilis* type produced by recombination (previously reported by the writer) (*2*) may be explained by the interaction of two factors for type of floret separation and their associated linked factors.

Abnormalities in the form of dwarfs were observed in five families. (Table 5.) Normal and dwarf plants occurred in proportions which indicate apparently regular ratios of 3:1 and 15:1. Judging by the types of segregation it is possible that dwarfing was due to definite factors, but this was not investigated sufficiently to justify definite conclusions.

TABLE 5.—*Breeding behavior for inheritance of type of floret separation, species types, and dwarfs in five F_3 families in the cross Coastblack × Avena fatua*

F_2 type sown	Type of floret separation and observed segregation	Calculated ratio	Dev. / P. E.	Number of plants		Calculated ratio of N:D	Dev. / P. E.	Total number of plants
				Normal	Dwarf			
Nonarticulate (byzantina).	35 homozygous byzantina.	.....	.....	35	13	3:1	0.50	48
Do	37 byzantina to 7 sterilis	3:1	2:06	44	17	3:1	.77	61
Do	33 byzantina to 11 fatua	3:1	0	44	5	15:1	1.70	49
Do	22 byzantina to 18 sterilis to 4 fatua (χ^2= 14.55; P=0.0007).	12:3:1	.....	44	2	15:1	.79	46
Articulate (fatua)	34 homozygous fatua	.....	.....	34	4	15:1	1.61	38
Total								242

The expression of the awned character was found to overlap in the four crosses in which awns were studied in detail. Plants having absolutely awnless panicles produced mostly awnless families, but occasional plants developed a few awns. A few plants classed as awnless in the two crosses North Finnish × *Avena sterilis ludoviciana* and *A. sterilis ludoviciana* × Probsteier produced families whose plants were completely awned. A few plants classed as partly awned in the

cross North Finnish × *A. sterilis ludoviciana* produced families having awnless plants.

Plants with strongly geniculate and twisted awns on the first floret on all spikelets segregated both for type of floret separation and type of awn. Plants with strongly geniculate and twisted awns on the two lower florets always bred true for similar awns and the wild-type floret separation, either *fatua* or *sterilis*.

Hairiness of Lemma

A 1-factor difference for hairy and glabrous lemma was shown by the occurrence of homozygous parent-type families and families segregating in a ratio of 3 hairy to 1 glabrous in the cross *Avena fatua* × *A. sterilis ludoviciana*.

Hairiness of lemma was studied in the F_3 of other crosses, but the families grown contained too few individuals to justify conclusions regarding the apparently complicated relations found. There was an indication of the presence of two independent factors or inhibitors for hairiness of lemma on the first and second florets. A homozygous family having only the first floret hairy was found in Richland × *Avena sterilis macrocarpa*.

Color of Lemma

The results of the study of color of lemma in the F_3 of the cross *Avena fatua* × *A. sterilis ludoviciana* verified the presence of a single-factor difference suggested by the F_2. Twenty families were grown. Seven were homozygous grayish white; 2 were homozygous brown; and 11 segregated into approximately 3 brown and light brown to 1 grayish white. The total of observed numbers for all families was 309 to 127 ± 18.3 with a $\frac{\text{Dev.}}{\text{P. E.}}$ of 3, which is at the boundary of the customary limit of error.

CORRELATION OF CHARACTERS

Floret Separation and Hairiness of Lemma

The correlation of several character pairs was studied. Independent segregation was found between type of floret separation and pubescence of lemma in *Avena fatua* (hairy) × *A. sterilis ludoviciana* (glabrous). A segregation of approximately 9 nonarticulate and hairy to 3 nonarticulate and glabrous to 3 articulate and hairy to 1 articulate and glabrous was obtained in F_2. (Table 6.)

Table 6.—*Segregation for floret separation and hairiness of lemma in F_2 of the cross Avena fatua (both florets hairy) × A. sterilis ludoviciana (both florets glabrous)*

	Nonarticulate (sterilis)		Articulate (fatua)		Total plants
	Hairy	Glabrous	Hairy	Glabrous	
Observed	245	90	68	27	430
Calculated (9:3:3:1)	241.9	80.6	80.6	26.9	
Deviation ($\chi^2=3.1061$; P=0.38)	3.1	9.4	12.6	.1	

By applying the χ^2 method for goodness of fit the value for P was found to be 0.38, which indicates a very good fit. (Table 6.) When the F_3 families segregating for both characters were combined, the value of P was 0.001, which indicates a poor fit. However, in the F_3 the families were small, and somewhat lower viability was evident in the *sterilis* segregates.

Spikelet Separation and Presence of Awns

Table 7 presents data on the F_2 of the crosses *Avena fatua*×Fulghum, *A. sterilis ludoviciana*×Probsteier, *A. sterilis ludoviciana*×North Finnish, and Richland×*A. sterilis macrocarpa*, showing the relation between type of spikelet separation, or disjunction, and the presence of awns on the lemmas of the first two florets of the spikelet. Complete linkage between the wild-type articulate spikelet and the presence of strongly geniculate and twisted awns on both florets was found in all crosses, except in *Avena fatua*×Fulghum, in which four *sterilis* plants appeared. Similar complete linkage was found in the F_3 of all crosses with the exception of *A. fatua*×Fulghum, where one *sterilis* plant was found in each of three families and two *sterilis* plants in a fourth family.

Spikelet Separation and Hairiness of Callus

Complete linkage was observed between spikelet separation by abscission as in *Avena fatua* and *A. sterilis* and the hairiness (ring of dense, more or less short thick hair) of the callus. However, in the cross North Finnish (nonpubescent)×*A. sterilis ludoviciana* (pubescent) the hair around the callus scar (the callus resulting from separation by abscission) was very much reduced in most *A. sterilis* families and segregates of the F_3 generation. This appears to lend weight to the belief that this form of *A. sterilis* is intermediate between the wild hairy forms and the cultivated *A. byzantina*.

TABLE 7.—*Segregation for spikelet separation and awns in F_2 of four interspecific oat crosses*

Cross, spikelet separation, and species type	Awnless or weak to strongly geniculate awns on first floret only	Strongly geniculate awns on both florets
Avena fatua×Fulghum:		
Semiabscission (byzantina)	334	
Abscission (fatua)		140
Abscission (sterilis)		4
A. sterilis ludoviciana×Probsteier:		
Fracture (sativa)	508	
Semiabscission (byzantina)		
Abscission (sterilis)		192
A. sterilis ludoviciana×North Finnish:		
Fracture (sativa)	1,014	
Semiabscission (byzantina)		
Abscission (sterilis)		375
Richland×A. sterilis macrocarpa:		
Fracture (sativa)	370	
Semiabscission (byzantina)		
Abscission (sterilis)		131

Hairiness and Color of Lemma

Hairiness of lemma appears to be almost completely linked with brown color of lemma in the cross *Avena fatua* × *A. sterilis ludoviciana*. (Table 8.) In an F_2 family of 430 individuals, the 313 hairy plants of both the *sterilis* and *fatua* types had brown lemmas. One plant of the glabrous *sterilis* and *fatua* plants had brown lemmas. No rain fell on the harvested grain to cause brown stains before the plants were examined.

Similar results were obtained in the F_3 generation. Only one plant with brown lemmas was found among the glabrous plants of the eight families grown. (Table 9.)

Table 8.—*Segregation for hairiness and color of lemma in F_2 of the cross Avena fatua* × *A. sterilis ludoviciana*

Hairiness of lemma	Lemma color and species type			
	Sterilis		Fatua	
	Brown	White	Brown	White
Hairy	245		68	
Glabrous	1	89		27

Table 9.—*Segregation for hairiness and color of lemma in eight F_3 families of the cross Avena fatua* × *A. sterilis ludoviciana*

Hairiness of lemma	Lemma color and species type			
	Sterilis		Fatua	
	Brown	White	Brown	White
Hairy	172		88	
Glabrous	1	41		23

DISCUSSION

The types of floret separation found in the different species of oats studied here are conditioned by a unit factor in all crosses except *Avena fatua* × Coastblack. Floret separation by basifracture, as in *A. sterilis*, was dominant in the crosses *A. fatua* × *A. sterilis* and *A. sterilis* × *A. sativa*, as well as in the crosses *A. byzantina* × *A. fatua*. Spikelets with strongly persistent second rachilla segments were found in the F_1 of all these crosses. The manner of separation of the spikelet from the pedicel, by fracture, as found in cultivated oats, was dominant over spikelet separation by abscission, as found in both *A. fatua* and *A. sterilis*. Crosses between *A. fatua* × *A. sativa* were not studied by the writer, but Surface (*9*) and Love and Fraser (*6*) found that the *sativa* rachilla was dominant in such crosses. These investigators found that the type of floret separation was controlled by a single-factor difference. Tschermak (*10*), on the other hand, believes that he has found a 2-factor difference in hybrids of these two species.

The inheritance of type of floret separation observed in the F_2 progeny of Coastblack × *Avena fatua* indicated the presence of two factors for this character in Coastblack. The Coastblack type of floret separation, which occurs by basifracture, was dominant in F_1 over the nonarticulate type of floret separation found in *A. fatua*. The segregation in F_2 was very close to the ratio 15 by basifracture (12 *byzantina* and 3 *sterilis*) to 1 by disarticulation (*fatua*). Two factors for *sterilis* type of floret separation are assumed to be present in Coastblack, namely, *B* for *byzantina*, which also inhibits wild base and associated characters, and *S* for *sterilis*, which does not inhibit wild base and associated characters. The recessive factors *b* and *s* are assumed for the type of floret separation found in *A. fatua*. The factorial constitution of Coastblack then would be *BBSS* and of *A. fatua*, *bbss*. The F_1 would be *BbSs*.

The complex of characters, oval pitted callus at the base of the spikelet, hairy ring around the callus, hairy rachilla, and strongly geniculate awns on the two lower florets, is closely linked in *Avena sterilis* with the factor for *sterilis* rachilla. The factor *S* for *sterilis* rachilla in Coastblack is believed to be associated with the wild complex of characters found in *A. sterilis*. This is emphasized, as it is desired to distinguish between *byzantina* segregates and the *sterilis*-like segregates formed by recombination. The wild *sterilis*-like segregates found in the F_2 and F_3 generations of Coastblack × *A. fatua* were identified by these characters associated with *sterilis* rachilla. The true-breeding *sterilis*-like form has been called "synthetic" *Avena sterilis*(2).

TABLE 10.—*Genotypes and phenotypes observed in F_2 and the theoretical expectation in F_3*

Genotypes	F_2 phenotypes	F_3 expectations
1 BBSS	Avena byzantina	True breeding.
2 BbSs	do	Do.
2 BbSS	do	3 byzantina to 1 sterilis.
4 BbSs	do	12 byzantina to 3 sterilis to 1 fatua.
1 BBss	do	True breeding.
2 Bbss	do	3 byzantina to 1 fatua.
1 bbSS	A. sterilis	True breeding.
2 bbSs	do	3 sterilis to 1 fatua.
1 bbss	A. fatua	True breeding.

The presence of moderately well-developed awns on both lower florets and a fairly large callus with its associated hairs in Coastblack suggests that the character expression of the factor complex for *byzantina* is modified by the *S* factor or factor complex. The *S* factor or group of factors in Coastblack is believed to be capable of producing the usual *sterilis* complex of spikelet characters. The synthetic *Avena sterilis*, accordingly, would have the formula *bbSS*.

The ratio 3 *byzantina* to 1 *sterilis* found in F_3 must have arisen from a zygote *BbSS*, in which the factor *B*, when present in either single or double dose, is capable of preventing expression of the strongly geniculate awn and articulate spikelet by the factor *SS*. The *byzantina* zygote *Bbss* would yield a ratio of 3 *byzantina* to 1 *fatua;* the *sterilis* zygote *bbSs* would give 3 *sterilis* to 1 *fatua*.

Families of these various types and families like F_2, as well as true-breeding families of *byzantina*, *sterilis*, and *fatua*, were obtained

in F_3. The wild forms were equipped with densely haired lemmas in most cases. The *byzantina* families were variable in this respect. A number of the *sterilis*-like plants were observed to have an awn on the third or middle floret of the spikelet. In most forms of ordinary *Avena sterilis* the third floret is awnless, while in *A. fatua* it usually is awned. This may be considered at least as partial evidence of the presence of an *A. fatua* factor in the synthetic *A. sterilis*.

In the F_2 progeny of Coastblack × *Avena fatua* the segregation of 15 nonarticulate rachillas to 1 articulate rachilla shows that two similar factors for this character are present in Coastblack. The results obtained in the F_2 and F_3 generations show that both factors produce approximately the same type of rachilla, but one is linked with a factor complex producing cultivated base and its associated characters, while the other is linked with a factor complex producing wild base and its associated characters.

The occurrence of a homozygous *sterilis*-like form in the cross Coastblack × *Avena fatua* suggests a number of interesting recombinations in crosses. The homozygous *sterilis* segregate *bbSS* crossed with the homozygous *byzantina* segregate *BBss* should give a hybrid progeny similar to that obtained in Coastblack × *A. fatua* with an F_2 ratio of 15 nonarticulate (*sterilis*) to 1 articulate (*fatua*). Fulghum crossed with the same *sterilis* form likewise might be expected to give similar results. Fulghum crossed with Coastblack would give only *byzantina*, since both varieties are homozygous for a *byzantina* pair of factors.

Natural crossing between Coastblack or similar varieties and *Avena fatua*, where these species are found growing side by side, could account for some of the *sterilis*-like forms found in nature.

SUMMARY

The inheritance of floret separation and associated characters was studied in seven interspecific oat crosses. The species used included varieties of *Avena sativa*, *A. byzantina*, *A. fatua*, and *A. sterilis*, all belonging to the 42 ($2n$[6]) chromosome group. Type of floret separation was governed by a unit factor in two crosses of *A. fatua* × *A. sterilis*, three of *A. sterilis* × *A. sativa*, and one of *A. fatua* × *A. byzantina*. *Sterilis* rachilla was dominant in F_1 in all crosses.

A 2-factor difference for type of floret separation was found in the cross *Avena byzantina* var. Coastblack × *A. fatua*. The segregation in F_2 was 15 nonarticulate to 1 articulate. Classified as to species types, the segregation was 12 *byzantina* to 3 *sterilis* to 1 *fatua*. The factor relationships suggested by the F_2 data were verified in the F_3. The true-breeding families included a new recombination *sterilis*-like type which has been called synthetic *A. sterilis*. Heterozygous families included those segregating into 3 *byzantina* to 1 *sterilis;* 3 *byzantina* to 1 *fatua;* 3 *sterilis* to 1 *fatua;* 12 *byzantina* to 3 *sterilis* to 1 *fatua*.

Both brown and grayish-white lemmas and hairy and glabrous lemmas were controlled by a unit factor in the cross *Avena fatua* × *A. sterilis ludoviciana*. Brown color and hairiness of lemma were dominant in the F_1.

[6] $2n$=diploid chromosome number.

Type of floret separation and hairiness of lemma segregated independently in the cross *Avena fatua*×*A. sterilis ludoviciana*. Strongly geniculate and twisted awns and *fatua* (hairy) rachilla apparently were completely linked in all crosses with the exception of *A. fatua*×*A. byzantina* var. Fulghum. In this cross slight crossing over occurred, which produced wild *sterilis* and also what appeared to be *sativa*-like plants. Strongly geniculate and twisted awns and hairy rachilla apparently remained completely linked in *A. sterilis*. Strongly geniculate and twisted awns on both lower florets were completely linked with hairy rachilla (dorsal side) in the wild forms. Hairiness of lemma was almost completely linked with brown color of lemma in the cross *A. fatua*×*A. sterilis ludoviciana*.

The wild-oat complex of associated characters consists of spikelet articulate in a large oval pitted callus surrounded by a ring of short stiff hairs, hairy rachilla, and strongly geniculate and twisted awns on the first two florets. This character complex is controlled by two or more closely linked factors. Solidified spikelet attachment and awns weak or nearly wanting are associated characters in the cultivated varieties.

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